

LABORATORY DIAGNOSIS

Diagnostic Form of Malaria Parasites

All are drawn from preparations stained with Wright's stain to a scale corresponding to a magnification of 2500 diameters. Hence, they are directly comparable with the other colored hematologic reproductions in this text. The diameters given in micra apply to the red cell containing the parasite rather than to the parasite itself.

1 to 4 The *Plasmodium vivax* of (benign) tertian malaria

5 and 6 The *Plasmodium malariae* of quartan malaria

7 and 8 The *Plasmodium falciparum* of estivo autumnal, tropical, malignant tertian, or subtertian malaria

3, 7, and 8 are sexual forms or gametocytes, the others are asexual forms

Note the character of the blue of the cytoplasm and red of the chromatin which is the same in all forms and entirely different from the slightly purplish blue staining of blood platelets

1 Ring form (about 8 hours after the chill) Note the normal size and staining of the red cell. Estivo autumnal rings differ only in being slightly more delicate, in a greater tendency for the chromatin mass to project externally from the circumference of the ring and in the more frequent occurrence of two or three rings in one red cell. Quartan rings are indistinguishable, but average slightly thicker than the tertian.

2 Young pigmented form (about 24 hours after the chill) Note that the red cell is now definitely enlarged and contains reddish yellow Schueffner's granules. Brownish pigment granules have appeared in the parasite, which at this stage may be of any imaginable shape.

3 Female sexual form (macrogametocyte) Note the large size and pale color of the red cell, and the pigment granules in the parasite. The shape and location of the chromatin is characteristic of the sexual form. A schizont of this size would be in the presegmenting stage and show the chromatin divided up into many separate masses similar to 6, a quartan presegmenter.

4 Segmenter (seen at the time of the chill or just before) Note the number of segments (12 to 24), most often 16 as here shown, and the large size of the red cell which is now very pale. The form of several of the separate segments is distinctly visible and is identical with that of the hyalin form which these segments are called as soon as they burst from this cell to enter another and repeat the cycle.

5 Quartan band form (about 30 hours after the chill) Note the smaller size of the red cell than in the corresponding tertian stage (2), the deep brassy color of its cytoplasm with no Schueffner's granules, and the characteristic band or oval form of the parasite.

6 Quartan presegmenter (about 70 hours after the chill) Note again the small size of the red cell and its deeper staining as compared to the tertian form, and the smaller number of segments which are about to form (6 to 12), usually as in this instance 8.

7 and 8 Estivo-autumnal ovoid and crescent (gametocytes) The crescent form is much more characteristic and more often seen. Note the pale segment of a red cell visible on the concave surface. The red cell is often extremely difficult to see or entirely absent. These forms if found, can scarcely be mistaken for any other type of parasite. Of the asexual stages only the ring forms usually appear in the peripheral blood and these may be difficult to differentiate from the tertian ring (1).



76u
1



88u
2



104uX88u
3



10u
4



72u
5



68u
6



64uX76u
7



8

A Textbook of
LABORATORY
DIAGNOSIS

*With Clinical Applications for Practitioners
and Students*

by

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CHILDREN PORTLAND OREGON

THIRD EDITION

+
WITH
TWENTY-SEVEN FIGURES IN THE TEXT
AND TEN COLORED PLATES



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Dedicated

IN APPRECIATION TO THE MEMORY
OF

HOWARD D HASKINS

Co-author of the First Edition

Whose Life as Teacher, Friend, and Co-investi-
gator was a Constant Example of Intellectual
Integrity and Unselfish Interest in the Success
of his Students

PREFACE TO THE THIRD EDITION

Progress in the field of laboratory diagnosis has made necessary a thorough line by line revision of the text with changes on almost every page. The general plan of the book as outlined in the preface to the first edition has been retained. The text has been increased by sixty eight pages and the indexes considerably enlarged, notwithstanding deletions of obsolete material and the use of fine print for the less frequently used discussions.

Rearrangement of some of the chapters was made to conform to the usual teaching program, but the chapters still may be covered in any order desired by the instructor since they are units in themselves. So much was added to the discussion of hematology that it seemed desirable to divide this into three chapters and to rearrange the material so that all data pertinent to the disorders of erythropoiesis, of leukopoiesis, and of the hemostatic mechanism, would be found together. The chapter on disorders of the ductless glands now follows the chapter covering disorders of carbohydrate, protein and fat metabolism. The chapter dealing with disorders of the central nervous system and the differential diagnosis of coma now follows the chapters on hematology and disorders of the respiratory and cardiovascular systems. The chapter on pregnancy has been placed at the end of Part One since this will serve as a review of most phases of laboratory diagnosis discussed in the preceding chapters.

Topics not covered or that have received fuller discussion in this edition than in previous editions include hemoglobinemia, methemoglobinemia, sulphemoglobinemia, the Addis sediment count, plasma and serum proteins, the laboratory diagnosis of endocrine disturbances, liver function tests, the nomenclature of cells of the blood and blood forming organs, the unavoidable error in cell counting methods, and the photoelectric colorimeter. The discussions of the differential diagnosis of anemias and leukemias have been largely rewritten. A discussion of the differential diagnosis of anemias in infants and children is included. The original tables designed to aid in the identification of cells of the blood and bone marrow, first published in the *Atlas of Hematology* by Osgood and Ashworth, are an important addition.

New standards for the normal total, differential and absolute leukocyte counts for different age and sex groups, based on data collected over a period of years, are given. A revised classification of leukocytoses and leukopenias is presented. Brief mention is made of the general principles which govern the indications for and the interpretation of bacteriologic and serologic methods, but no attempt is made to make this book a substitute for a textbook of bacteriology. The chapter on the disorders of the central nervous system has been almost completely rewritten with a much more complete discussion of the differential diagnosis of coma.

New methods, the indications for, interpretation and technic of which are included for the first time, are the one hour two dose dextrose tolerance test, a simple quantitative method for urobilinogen in urine and feces, quantitative methods for the determination of sulfanilamide and sulfapyridine in blood and urine, simple original methods for the quantitative determination of carbon monoxide hemoglobin and methemoglobin in blood, quantitative methods for the estimation of vitamin C, and the determination of the prothrombin time as a guide to vitamin K deficiency. The technics of lumbar and cisternal punctures are described. Recently developed and more accurate standards for basal metabolic rate determination in children are tabulated. Minor improvements in the technics of many methods are included. A system of urinalysis for rapid and efficient examination of a large number of specimens is outlined. The Index by Diseases as well as the Subject Index has been thoroughly revised and brought up to date to enable the intern and busy practitioner in any field of medicine to find with a minimum expenditure of time the laboratory aids to diagnosis, prognosis and treatment which are worthy of consideration in any patient.

In revising the references care was used to follow the criteria of selection given in the preface to the first edition in order that they might be as useful as possible to the student, intern, or physician using the book. An Author Index has been included to facilitate finding desired references.

A new illustration of organic sediments in urine replaces the former figure 11.

I wish to thank the many teachers of laboratory diagnosis who are using this book as a required text, and especially Dr Howard L. Alt of Northwestern University Medical School for many helpful suggestions. I am grateful to the following investigators and their publishers, among others, for permission to include methods, standards, and results of their studies: S. E. Gould, R. Sparkman, H. H. Merritt and F. Fre

mont Smith, F B Talbot, C J Farmer and A F Abt, E K Marshall, Jr and J T Litchfield, Jr, and A J Quick I am indebted to Dorothy Madge Ellis, Rachel Ellis, and Mrs Mable Wilhelm Osgood for much assistance in the preparation of this revision, and to Miss Clarice Ashworth for drawing figure 11 I also want to thank the publishers for their hearty cooperation

EDWIN E OSGOOD

PORTLAND OREGON

PREFACE TO THE FIRST EDITION

This book is an outgrowth of the outline which has been used for some years in teaching laboratory diagnosis (clinical pathology) at the University of Oregon Medical School. Its object is to teach the habits of thinking that are necessary to obtain the fullest information from laboratory sources, using as a basis for this teaching the body of knowledge which it is essential for every graduate of a modern medical school to possess. We shall indeed feel happy, if, in addition, we have succeeded in making the fascination and daily usefulness of this subject so obvious that at least some of our readers will pursue their study of it, not merely to the end of this or any other book, but throughout their lives.

The subject matter has been divided into its two natural divisions. Part One is a consideration of that body of knowledge which the practitioner must have available at the bed side of the patient. It gives information which will aid in answering such questions as the following: What laboratory procedures will aid me in making the diagnosis on this patient? Are any of these procedures contraindicated? How often should they be repeated? What am I, and what am I not, justified in concluding from this laboratory report? Is my treatment of the case producing the desired result? Is my treatment doing harm?

Part Two is a consideration of that body of knowledge which it is necessary to have available in the laboratory. It will aid in answering such questions as the following: Which method am I to choose for this determination? How much material is required and how should it be secured and conveyed to the laboratory? How do I make this estimation or reagent, how identify this cell, crystal, or ovum? What are the limits of error in this determination? How may the common mistakes in technic be avoided? How can I determine whether my technic is satisfactory?

The arrangement of the subject matter by systems (see Table of Contents) has been found by actual experience to increase very greatly the student's interest in the work. If a test is presented to him merely as a part of the examination of the urine he may be frankly bored. However, if the same test is presented as an aid in the diagnosis of disorders of the kidney or urinary tract, his interest is at once aroused, as he knows he will have such diagnoses to make. The practitioner's

problems arise in the same way. He wishes to know how to arrive at a diagnosis in a particular type of case. In this text he can find an organized discussion of the laboratory phase of this diagnosis in the chapter treating of that system, in some instances together with tables of differential diagnostic points.

Each chapter is introduced by the briefest possible resume of the essential anatomy, physiology, biochemistry, and pathology of the system under consideration, the object of which is to correlate this subject with knowledge the reader already has and to stimulate him to apply it. Of necessity these introductions are extremely incomplete, but a few references are given with each which will guide the interested reader to fuller expositions.

Theoretical discussions are beyond the scope of a work of this type but we have tried to label fact and theory clearly, and to indicate the most important points of controversy with a reference or two.

The separation of the information needed in the laboratory from that needed at the bedside permits the individual interested either in technic or in interpretation to study as a connected whole the phase of the subject which interests him more.

The Index by Diseases of diagnostic measures in addition to the usual index, should not only serve as a valuable outline for review by the student but should also make this a handy reference volume for the busy practitioner.

No effort has been made to have the list of laboratory tests for a given determination exhaustive. We give for each determination the method which in our experience is preferable, and a critical discussion of such others as are so commonly used as to require mention. The fact that a method does not appear in this book does not necessarily imply that it is not good. To include all the methods which are in use, would have increased the bulk of the book to an extent which would preclude its being covered, as a whole, in the time allotted for the usual course in laboratory diagnosis, and would be a duplication of numerous texts now on the market. We do not pretend to advise the trained chemist or the experienced teacher as to which method to use, but we do wish to acquire the reputation of having, as the recommended method for each determination in the most recent edition of this book, a test which is as satisfactory for clinical purposes as any available at the time of writing, which has been personally tested in hospital, dispensary, and laboratory, and which will, therefore, be a safe choice for those who do not have the time and facilities necessary for proper evaluation of new methods.

All but the simplest bacteriological and serological methods have been omitted, as these subjects are adequately considered in many excellent texts and are given in separate courses in the medical curriculum. However, the chief indications for bacteriological and serological procedures have been included in the Index by Diseases.

With each recent method a reference is given to the original source. This is our acknowledgment of indebtedness to the author. The methods are given in Part Two as we recommend doing them and contain many minor modifications which extensive use has led us to believe are improvements. We assume full responsibility for the changes made, and want it clearly understood that the authors of the method are responsible only for the technic as given in their original articles and have not been consulted as to the technic as given here. We have made an earnest effort to give proper credit. If we have failed at any point, it has been through oversight, and we will appreciate having it called to our attention.

The bibliography has been planned with the following objects in view: first, to familiarize the reader with the names of a few of those who may be expected to contribute further outstanding work in the field under discussion and with the periodicals in which it is most likely to be found; second, to supplement and extend the information given here and to give points of view differing from our own; third, to give good examples of each type of medical writing pertaining to laboratory diagnosis; fourth, to give wherever possible, references to reviews with good bibliographies which will guide the reader to the important literature with a minimum expenditure of time; and fifth, to limit the bibliography as far as possible to material which is not more easily accessible in the standard textbooks. Hence, the references are largely to recent literature and will require revision from time to time. With the object of saving the reader's time, references have been given as fully as is possible, and, while many important ones are omitted, no reference has been included which will not repay the time spent in reading it. The bibliography has been chosen for the reader, and not with the object of giving the sources of all the information included. A complete bibliography of all the references consulted would almost double the size of this book. Hence it must suffice if we here acknowledge our indebtedness to some extent at least, to nearly everyone who has published work in this field in the last fifteen years, and particularly to those who have supplied us with their reprints.

To Frank I. Trotman we are indebted for assistance in preparing Part Two on laboratory methods for many valuable suggestions and

for help with the proof-reading. In addition we wish to thank Mrs Grace H Osgood and Miss Mable M Wilhelm for much help in the preparation of the manuscript and the proof reading. We are indebted to many members of the faculty of the University of Oregon Medical School for advice, but in particular we are grateful to Dr Laurence Selling, head of the Department of Medicine, for suggesting improvements in Chapter V and for his constant interest in the progress of the work.

The junior author (E L O) wishes, also, to acknowledge his indebtedness both for information and inspiration to his teachers on the faculties of the University of Oregon and Vienna, and on the medical staff of the Mayo Foundation.

We wish here to give credit to Miss Clarice Ashworth for her remarkable art work without which the original colored illustrations so skillfully reproduced by the Hicks Chatten Engraving Company would have been impossible, and to acknowledge our gratitude to our publishers, P Blakiston's Son and Co , Inc , for their liberal publication policy, hearty cooperation, and excellent treatment of our work.

EDWIN E OSGOOD
HOWARD D HASKINS

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PART ONE

at the outset that laboratory diagnosis is largely applied biochemistry, physiology, and pathology, that bacteriology, parasitology, and serology are but subdivisions of laboratory diagnosis, and that as an integral part of the study of the patient, laboratory diagnosis is part of the clinical practice of medicine and necessary as well in any specialty. It is most important of all to keep in mind that the laboratory study of the patient is a valuable supplement to, but not a substitute for, a careful history and a thorough physical examination.

II GENERAL PRINCIPLES¹

A Only generalizations can be given in books, while clinical practice is a series of individual problems. Blind following of this or any other text will be of little profit. Exceptions will be found to most rules in medicine, those in this textbook included. Regard even the most didactic statements which occur in the following pages as points of departure in *thinking rather than as ultimate goals*. I have tried to recommend laboratory procedures in the various types of cases that would constitute an ideal study of a case from the standpoint of the patient's physical welfare, but the patient's economic, social, and mental well being must also be taken into consideration. The laboratory examinations which the physician can conscientiously recommend are those which he, still possessed of his own knowledge of the disease and of the tests available, would want done on himself, were he in the patient's physical, economic and social position, and were he required to pay the price expected of this patient for this quality of laboratory work.

This artifice of putting one's self in the patient's place will simplify decisions on many other phases of medical practice, not the least important of which is the decision as to the desirability of consultation with an expert in some special field. In considering the benefits to be derived by the patient from such consultation, the special knowledge of the expert in laboratory diagnosis should not be overlooked.

B *Planning the Laboratory Study*—To conserve time and expense for the patient and give maximum aid to the physician, a definite effort should be made to plan all the laboratory work which is indicated immediately on *completion* of the history and physical examination while the data are freshly in mind and the patient is still available. Should venipuncture, spinal puncture, etc., be needed. It is a common mistake to wait for the results of a urinalysis, blood count, or Wassermann test when a moment's thought would make clear the necessity.

¹ Kilduffe R. A. The Clinical Utilization of Blood Studies. J. Lab. & Clin. Med. 922-937 (June) 1938.

or other tests, for instance, a blood sedimentation rate determination, spinal fluid examination, or an icterus index estimation, irrespective of what the results of the first mentioned tests might be. This delay sometimes means the loss of lives and often the loss of days of valuable time.

As each patient is studied, give definite thought to the chemical and physiologic changes underlying the signs and symptoms observed, and plan the laboratory examination with the object of securing a clear mental picture of these fundamental changes. Have a definite reason for ordering or performing each test. If this plan is followed, physicians will not display their ignorance by such absurd requests as for a "complete blood count" or a "complete blood chemistry."

Any important deviation from normal in the results of a laboratory examination should be followed by repeated tests until the results have either returned to normal and the tendency to deviate has disappeared or the patient has died.

C Interpretation of Laboratory Data—In interpreting results, transform laboratory data first into a mental picture of the fundamental pathologic, chemical and physiologic changes which could explain them, and, having these clearly in mind, consider all the causes which may produce these changes, giving due weight to their relative frequency. It is much easier to interpret hematuria if we first think of it as due to a hemorrhagic inflammation or an ulceration somewhere in the urinary tract, or to one of the systemic conditions associated with a tendency to hemorrhage, and then analyze each of these subdivisions further, than if we simply try to remember by rote all the causes of blood in the urine.

Keep observations clearly separated, in records and thinking, from the conclusions or hypotheses that have been formulated from these observations. Observations are much more apt to be correct than the conclusions based on them. Hippocrates would have but little fame today if he had recorded only his conclusions. We all have a great tendency to go beyond our facts. How common is such thinking as the following: The patient has a red cell count of 250 million. Ova of the beef tapeworm are found in the feces. Therefore, his anemia is due to infestation with tapeworms. Actually, we are justified in concluding only that, if no errors in identification or technic have been made, and no errors in labelling of the specimens have occurred, the patient has anemia and is harboring at least one tapeworm. Whether the worm is, in part or in whole, responsible for the anemia must still be determined. A patient particularly if past middle life, may have

tion is desired than is found where the test or disease is first mentioned. If the name of a disease means little or nothing to you, do not proceed until it has been looked up.

To learn the interpretation of laboratory findings, first master the broad general statements which cover the fundamental causes. Then, as a rule, your knowledge of pathology will suggest the subdivisions.

When the assignment has been covered in this manner, return to these suggestions for study and follow them through mentally, without referring to the body of the text. When finished, refresh your memory again on the things which had been forgotten. Repeat this until the subject matter of the assignment is well in mind.

Acquire the habit of refreshing your memory on the laboratory phase of each disease as it is studied in medicine, surgery, or the specialties and in connection with each patient that you see. Use the Index by Diseases in working out differential diagnoses.

To keep up to date, it will be necessary to learn to utilize the literature of this subject. This, like all textbooks, must be somewhat behind the forefront in some phases, therefore, not only make sure that you have available for reference the most recent edition of one or more good supplementary books,¹ but get in the habit of reading critically the current literature on this subject.

¹ Of the more extensive reference books on laboratory diagnosis available at the time of writing I would recommend Peters J. P. and Van Slyke D. D. *Quantitative Clinical Chemistry*. Vol. I and II. The Williams and Wilkins Co., Baltimore, 1931.

² Much of it is scattered but for the American medical student. *The Archives of Internal Medicine*. *The American Journal of Clinical Pathology*. *The Journal of Laboratory and Clinical Medicine*,³ and the *Journal of Biological Chemistry*,⁴ offer the most easily accessible concentrated sources.

CHAPTER II

DISORDERS OF THE KIDNEY AND URINARY TRACT WITH ESPECIAL REFERENCE TO NEPHRITIS

I RESUMÉ OF THE ESSENTIAL POINTS IN THE ANATOMY, PHYSIOLOGY AND BIOCHEMISTRY OF THE FORMATION OF THE URINE¹

The functional unit of the kidney is the glomerulus with the tubule leading from it to the collecting tubule. The glomerulus consists of a hollow sphere of very thin epithelium, opening into a tubule, which is invaginated by a tuft of capillaries. The tubule is lined with thicker epithelium and is divided into a proximal convoluted portion, the loop of Henle and a distal convoluted portion. The blood supply of the tubule is a second group of capillaries arising from the efferent vessel of the glomerulus. Therefore, it is supplied only by blood which has already passed through the glomerulus, the composition of which is certainly different from that on either the arterial or the venous side of the circulation. For the same reason any interference with the blood supply of the glomerulus must of necessity affect the tubule also, and any toxin carried by the blood stream that reaches the glomerulus is almost certain to come in contact as well with the corresponding tubule. No toxin can reach the tubule through the blood stream without first reaching the glomerulus. Hence the burden of proof of the existence of separate lesions should rest on those who believe them to occur.

The mechanism of the formation of urine is not definitely settled. The theory¹ having the most support is that in the glomeruli a dialysate of the plasma is formed, from which, on passing through the tubules, the threshold substances are reabsorbed. The extent and nature of this process can best be illustrated by Table 1 which is modified from Cushny.

The function of the kidney is best understood if it is regarded primarily as an organ whose work is to maintain the chemical and physical qualities of the blood plasma within normal limits. In this function it is greatly aided by the lung which regulates all of the gaseous interchanges and excretes certain volatile drugs and poisons, and to a less extent by the sweat glands, liver and bowel which function in a manner similar to the kidney but much less effectively. Once this view becomes clear one sees that the formation of the urine is merely a by-product of such kidney activity and one realizes why the urine is so extraordinarily variable in composition while the plasma remains so uniform.

¹ Richards A. N. *Urine Formation in the Amphibian Kidney*. The Harvey Lectures. Vol. 30 pp. 93-118. Williams and Wilkins Co. Baltimore 1936.
Cushny A. K. *The Secretion of the Urine*. 2d ed. Pp. 283. Longmans Green and Co. London 1926.
Wiggers C. J. *Physiology in Health and Disease*. Pp. 831-830. Lea and Febiger Philadelphia 2d ed. 1923.

TABLE 1 *—URINE COMPARED WITH PLASMA AND GLOMERULAR FILTRATE (CUSHNY)

	Plasma	Glomerular filtrate, 83 liters contains	82 liters reabsorbed fluid contains		1 liter of urine produced contains		Change in concentration due to kidney action
	Per cent	Grams	Per cent	Grams	Per cent	Grams	
1 Colloids	70						
2 Water†	920				950		
3 Glucose†	01	900	011	900			
4 Bicarbonate†	015						
5 Sodium†	030	2700	032	2665	035	35	10
6 Chlorine†	037	3330	040	3270	060	60	20
7 Potassium†	002	180	002	165	015	15	70
8 PO ₄ †	0009	81	00008	66	015	15	160
9 Uric Acid	0002	18	00016	13	005	05	250
10 NH ₄	00017	09	000061	05	004	04	400
11 Urea	003	270	0008	70	200	200	600
12 Creatinin	0001	09	000018	015	0075	075	750
13 SO ₄	0002	18			018	18	900

* Modified from Cushny The Secretion of Urine pp 16 and 57

† Threshold Substance

The kidney is influenced in its function only by excess or deficiency of any particular substance in the plasma reaching it, not by excess or deficiency of that substance in the body as a whole, except as it modifies the composition of the plasma. Thus, marked edema may be perfectly compatible with deficient water excretion by a kidney perfectly capable of adequate fluid output if that fluid should be presented to it as excess in the plasma.

A normal urine is, therefore, not susceptible of accurate definition even if the type and amount of diet and the fluid intake are known, for variations in external or internal temperature (sweating), in degree of physical activity, and physiologic variations in metabolism also influence its composition. Only when all these factors are known or, better, when the exact composition of the plasma reaching the kidney is known, can an even reasonably accurate prediction of the urine formation by the normal kidney be made.

The functions of the kidney include the retention of colloids in the plasma, the maintenance of water balance, the maintenance of acid-base balance,¹ in which it is aided by the lungs, the removal of waste products, the non-threshold substances (Table 1), drugs and poisons, the maintenance at normal levels of the threshold substances (Table 1), and the maintenance of the normal osmotic pressure of the plasma. In addition the kidney is able to synthesize hippuric acid from benzoic acid and glycine, to form ammonia from urea when it is needed, and to hydrolyze organic (hexose) phosphate compounds to inorganic phosphates. The composition of the urine, aside from extraneous elements which may be

¹ This is more fully discussed in Chapter III

added to it after its formation, is, therefore, an indicator of the problems presented to the kidney by the plasma reaching it. Increases in the non-volatile constituents of the plasma not attributable to unusual intake or metabolism are the best indexes of impaired renal function.

Goldblatt and others¹ have demonstrated that renal ischemia results in hypertension. The hypertension occurs whether the ischemia is unilateral or bilateral, and is independent of the nerve supply, but is due to the formation of a substance (renin) in the kidney which, after reaction with a plasma factor, directly produces arteriolar constriction in all areas supplied by blood which has passed through such a kidney. This undoubtedly accounts for the hypertension characteristic of many diseases of the kidneys and of hypertensive cardiovascular disease.

II ESSENTIAL POINTS IN PATHOLOGY²

So much remains unknown, and so much speculation has been done on such slight foundation that it will require very clear thinking to separate fact from fiction in the field of kidney pathology. Names have been given which imply knowledge of disease processes which we do not possess. It has not been proved that the conditions formerly called nephritis are all inflammations, or are even primarily kidney diseases. "Lipoid nephrosis" may well be due to a decrease in plasma proteins from a deficient formation or excessive excretion, rather than to degeneration of kidney tubules.

Glomerular nephritis may be only the participation of the glomerular capillaries in a general capillaritis. The primary contracted kidney of hypertensive cardiovascular renal disease is almost certainly only one of the late results of a systemic condition.

There are certain broad pathologic concepts which can be relied on, however.

First, there is sufficient reserve renal tissue, so that about 80 per cent of it must be destroyed or incapacitated before any detectable change in renal function occurs. Therefore, unilateral renal disease practically never, and patchy renal disease very rarely, will show change in renal function as determined from blood chemistry or examination of bladder urine. On the contrary even slight diffuse bilateral renal disease will produce such changes.

Second, renal function depends on the blood supply to the kidney. Any alteration in the amount, composition, pressure, or physical properties of the blood reaching the glomerulus will be reflected in the urine formed, and such changes do not necessarily imply that the kidney is primarily at fault.

Third, inflammation anywhere in the urinary tract will introduce into the urine the exudate characteristic of the type of inflammation, i.e., catarrhal (pus), serous (pus and protein), hemorrhagic (pus, protein, and blood). Therefore, a review of the common inflammations of the urinary tract will aid greatly in the interpretation of these findings.

¹Goldblatt H. Experimental Hypertension Induced by Renal Ischemia. Harvey Lecture. Bull. New York Acad. Med. 14: 523-553 (Sept.) 1938.

Pickering G. W. and Prinzmetal M. Some Observations on Renin, a Pressor Substance Contained in Normal Kidney Together with a Method for Its Biological Assay. Clin. Sc. 3: 211-227 (Apr.) 1938.

²Montz A. R. and Oldt M. R. Arteriolar Sclerosis in Hypertensive and Non Hypertensive Individuals. Am. J. Path. 13: 679-728 (Sept.) 1937.

Fourth, in the male the ducts of the genital organs open directly into the urinary tract, and in the female the vaginal orifice is very close, therefore, secretions or exudates from these sources may introduce changes in the urine as voided

Fifth, casts are produced in the kidney tubules by reabsorption of the fluid from a protein-containing glomerular filtrate and the adherence to or imbedding in this hyaline mass of such cellular elements as are present in the lumen of the tubule. Therefore, the presence of casts means that some protein is entering the urinary tract at the glomerulus and that at least part of the disturbance lies here or proximal to this point. The opposite is not necessarily true.

Sixth, any partial obstruction in the urinary tract tends to produce hydronephrosis and, therefore, partial loss of function in the kidney affected. It also predisposes to infection in the urinary tract proximal to the obstruction which may result in further loss of function. Complete obstruction of a ureter produces atrophy, and, of course, total loss of function of the affected kidney. Therefore, a review of all the causes of obstruction in the urinary tract will be profitable.

III INDICATIONS FOR AND INTERPRETATION OF THE URINE EXAMINATION

The routine urinalysis as described in the laboratory section should be performed on every patient studied, as experience has shown that important discoveries not suspected from the history or physical examination are thus frequently made. This examination is doubly important in cases in which hypertension, diffuse edema (edema including the face in contradistinction to edema of passive congestion which begins in the most dependent portion), retinopathy, or local symptoms (smarting or frequent micturition, ureteral or bladder pain) focus attention on the urinary system. In such cases the total urinary output should be measured daily, to be compared with the record of the fluid intake. A routine urinalysis should be done on a properly preserved sample in acute cases daily and in chronic cases twice a week.

*A Heat Coagulable Protein Albumin and Globulin*¹—The presence of heat coagulable protein in the urine is called albuminuria, even though globulin is also present, usually in small amounts. If found, a rough quantitative estimation is desirable, but for most purposes the approximate quantitative expression of the results of the qualitative test is sufficient. The concentration (grams per liter) is more significant than the 24 hour excretion, but if the latter is great, this loss of body protein must be considered in planning the diet. Normal urine does not give a positive result with any of the usual tests.

The causes of albuminuria are as follows

¹ Keutmann E. H. and Bassett S. H. Studies on the Mechanism of Proteinuria. J. Clin. Investigation 16: 767-776 (Sept.) 1937

1 Nephritis of all types and all the conditions formerly called nephritis (p 44) The protein tends to be present in higher concentration in the acute stages An extremely high concentration (over 2 grams per liter) is a point in favor of the diagnosis of *lipoid nephrosis*, *amyloidosis*, or *sypilis* of the kidney In *chronic nephritis* and particularly in the primary contracted kidney of *hypertensive cardiovascular renal disease* only a one plus reaction is usually present and it is frequently absent for days at a time A similar trace of albumin is usually found in old persons without hypertension and is due to patchy lesions of senile atherosclerosis in the kidney A sudden increase in the heat coagulable protein content in chronic diffuse nephritis, especially if associated with increase in red cells and decreased daily volume, suggests an acute exacerbation A similar increase in coagulable protein in hypertensive cardiovascular renal disease usually means a superimposed passive congestion of the kidney due to heart failure

2 Febrile Albuminuria¹—In most febrile diseases a transient albuminuria occurs, associated with the changes in the kidney described by pathologists as cloudy swelling or parenchymatous degeneration There is also a tendency to oliguria (a small volume of highly colored urine of high specific gravity) These findings return to normal during convalescence However, a true nephritis may develop during the course of a fever This is especially apt to occur in scarlet fever, tonsillitis, sinusitis, other streptococcic infections, and diphtheria Therefore, the urine should be studied frequently and carefully in such cases

3 Passive Congestion of the Kidneys—This is a very common cause of albuminuria It is, therefore, important to recognize it so that it will not be confused with primary disease of the kidney Globulin often forms a greater proportion of the heat coagulable protein than it does in other forms of proteinuria It is most often due to congestive heart failure but any obstruction in the venous return from the kidneys to the heart will produce it The findings associated with the albuminuria in bilateral involvement are a small daily output of urine of high specific gravity, usually strongly acid in reaction and low in chloride content Crystals are usually present and in severe cases red cells also appear In cases uncomplicated by chronic nephritis or by impaired renal function of the type which occurs in some cases of hypertensive cardiovascular renal disease, urobilinogen is constantly present in increased quantities In cases with these complications a test for urobilinogen is negative and the specific gravity of the urine tends to be lower than would otherwise be expected

¹Wells J W Febrile Albuminuria Am J M Sc 1910 4 (July) 191,

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¹ Welty, J W Febrile Albuminuria *Am J M Sc* 194, 574 (July) 193

4 **Orthostatic (lordotic or postural) Albuminuria** ¹—This is probably only a subdivision of the preceding group as it is thought to be due to a congenital variation which permits partial obstruction to the return flow of blood through the left renal vein in positions which tend to increase the lordotic curve of the lumbar spine. It is not uncommon in young individuals. Since it rarely leads to serious consequences, it is of importance only because it must be differentiated from other types of albuminuria. The characteristic finding is the presence of albumin in the urine voided after the patient has been up and about, and its absence in urine voided after the person has been lying down. To test for it, have the patient bring a sample of urine voided immediately on arising in the morning. This should contain no heat coagulable protein. Then have the patient stand for half an hour with his back to a wall and his heels against it. This forces a lordotic position and urine voided at the end of this time should contain albumin. The best proof of the relation of the lordosis to the albuminuria is that albuminuria occurs when the patient is lying down if the back be forced into a lordotic position by a hard pillow under the lumbar spine.

5 **Physiologic (functional or cyclic) Albuminuria** ²—About five per cent of apparently healthy young adults show albuminuria. This is often transient, but may persist for years without the development of evident disease. In adolescence it is even more common. Over fifty per cent of persons will develop albuminuria after severe exercise. The appearance of albuminuria after cold bathing or the ingestion of large amounts of egg albumin has been reported. There is no satisfactory explanation for these physiologic albuminurias. Renal function tests should be repeated at intervals if albuminuria persists.

Note The great majority of cases of albuminuria seen clinically will fall in these first five groups.

6 **Complications of Pregnancy and the Puerperium**—Since albumin in the urine may be the first sign of an impending eclampsia, the urine should be tested frequently during pregnancy. On the other hand, albuminuria in pregnancy may be due to a passive congestion of the kidney from pressure of the uterus on the renal veins, to a true

¹Thorp E. G. and Wakefield E. G. Orthostatic Albuminuria. Comparison with Other Types of Albuminuria. *Ann Int Med* 6 1565-1578 (June) 1933.

²Diehl H. S., and McKinlay, C. A. Albuminuria in College Men. *Arch Int Med* 49 45 (Jan.) 1932.

Hellebrandt Frances A. Studies on Albuminuria Following Exercise I Its Incidence in Women and Its Relationship to Negative Phase in Pulse Pressure. *Am J of Phys* 101 357 (July) 1932.

Hellebrandt Frances A. Brogdon Elizabeth and Kelso L. E. A. Studies on Albuminuria Following Exercise II Its Relationship to the Speed of Doing Work. *Am J of Phys* 101 365 (July) 1932.

nephritis, to nephrosis, or to any other of the causes of albuminuria which may occur in a non pregnant woman. Further laboratory examination and the results of physical examination will make the differentiation.

7 Poisons—Temporary albuminuria following general anesthesia occurs in about one fourth to one third of all the cases and need not cause alarm.

Poisoning with the heavy metals, especially mercury, lead and bismuth, is common and results in chemical changes in the blood and urine similar to those in acute glomerular nephritis. The differentiation is made by the history and physical examination. If the patient does not die in the acute stage, complete recovery may be expected.

Turpentine, cantharides, or arsenic poisoning may produce a similar urinary picture.

It is important, therefore, to study the urine before and during the therapeutic administration of these drugs.

8 Trauma to the Kidney

9 Polycystic Kidneys—This is a rare congenital anomaly usually not becoming manifest until the patient is forty to sixty years of age. One to two plus albumin is usually found.

10 Severe Anemias—A one or two plus albuminuria is not uncommon and does not alter the prognosis.

11 Hemoglobinuria and Methemoglobinuria

12 Severe Obstructive Jaundice

In all of the above mentioned conditions pus cells are usually few or absent, and the albumin enters the urinary tract through the glomerulus so casts are usually present also. In the remaining types pus cells are numerous and albumin enters distal to the tubules so that casts rarely occur in uncomplicated cases.

13 Tuberculosis of the Kidney—This rarely causes albuminuria.

14 Pyelitis, Ureteritis, Cystitis, and Urethritis—Only unusually severe inflammations are apt to cause albuminuria. If casts and much albumin appear during the course of a pyelitis the serious complication of pyelonephritis or pyonephrosis should be suspected. Unfortunately, these complications may occur without producing albuminuria and casts are more often absent than present.

15 Pus or Blood—If in large amounts, these may be the sole cause of a one or two plus protein test.

16 Secretions or Exudates from the Genital Tract—These may be responsible for positive protein tests in voided urine.

The presence of proteinuria alone is never a sufficient basis for the diagnosis of nephritis, but the finding of albumin in the urine should always lead one to consider this possibility.

Bence Jones Proteinuria—This is rare but important. It is easily overlooked in the routine test for albumin unless the tests are performed as directed. It is found only in such extensive lesions of the bone marrow as the following

1 **Multiple Myeloma**—Most patients in whom Bence Jones proteinuria has been demonstrated have been proved to have these rare (Mayo clinic statistics show 15 cases out of 548 000 admissions) multiple bone tumors. About 20 per cent of cases of multiple myeloma, however, never develop Bence Jones proteinuria. It is possible to demonstrate the presence of this protein in the blood serum, and sometimes the total serum protein is increased. Of thirteen cases of multiple myeloma studied by the author, only two failed to develop Bence Jones proteinuria.

2 **Leukemias**—It has been reported in only a very small percentage of patients with these diseases. In a careful study of about one hundred cases I have never found it.

3 **Extensive Metastases of Malignant Tumors to Bone**—These have been reported to cause Bence Jones proteinuria. I have never found it in such cases.

The frequent association of Bence Jones proteinuria with anemia and renal insufficiency¹ usually without hypertension, has doubtless caused some cases of multiple myeloma to pass unrecognized under the diagnosis of nephritis.

C Other Proteins 1 **Proteose**—This is of little diagnostic value and of no value when albumin is also present. It occurs in any condition with extensive breaking down of cells, especially leukocytes, as in the absorption of an exudate resolving pneumonia, leukemia, abscesses and in severe liver disease. It may be absorbed from the intestine in ulcerative colitis, or occur in pregnancy due to absorption of amniotic fluid, or in the puerperium due to involution of the uterus. Proteose like substances from the seminal fluid or proteose resulting from the disintegration of pus or albumin in urine which has been allowed to stand several days may give rise to positive tests of no diagnostic significance.

The presence of an ether extractable substance described as urinary proteose by Ortel, has been reported as of significance in allergic conditions. Since this substance has been demonstrated in nonallergic conditions² and has been shown to give non specific skin reactions it is doubtful whether it will prove to be of clinical significance.

2 **Mucinous Proteins**, variously called mucin, mucoid, phosphoprotein, mucoprotein, nucleoprotein etc.—The chemical nature of this protein has not yet been determined. It appears in the urine in cases of severe cystitis and, perhaps pyelitis. It must be differentiated carefully from Bence Jones protein.

D Sediments—The examination of sediments is an extremely important part of urinalysis, too often slighted by medical students. It requires much controlled practice to learn to identify organic sediments correctly.

1 **Casts**—These are more important than albumin and may be found when the albumin test is negative. They indicate renal damage, although this may be only temporary, as in passive congestion of the kidney, or localized to a small area in the kidney, as in focal embolic nephritis. Red cell casts, leukocyte casts, and granular casts.

¹ Bannick E. G. and Green, C. H. Renal Insufficiency Associated with Bence Jones Proteinuria. Arch Int Med 44 486-501 (Oct.) 1929.

² Tuft L. and Brodsky M. Urinary Proteose in Allergic Conditions. J Allergy 4 534 (Sept.) 1933.

are most common in the acute inflammatory types of nephritis, hyaline in the chronic forms. Waxy casts are rare (said to be found in amyloid disease). Very broad casts (renal failure casts of Addis) indicate a bad prognosis, for they appear only in the last stages of severe renal disease.

Casts occur in all types of albuminuria in which the albumin enters the urine in the kidney and are absent in those types of albuminuria due to lesions lower in the urinary tract. They are usually numerous in cases with much albumin in the urine and vice versa, but may be present when the amount of albumin excreted is too small to give positive chemical tests. The causes are the same as those listed for albuminuria (A 1-12).

2 **Hematuria** ¹—This is blood as such in urine. An occasional red cell may be found in the urine of healthy persons, but one plus blood or over, not due to contamination from menstrual discharge, etc., is always significant. The amount of blood present is usually overestimated (p. 351). The loss of blood in the most severe cases of hematuria of nephritic origin probably rarely reaches 5 cc per day. The anemia of nephritis is, therefore, not due to hemorrhage. Other causes, particularly bladder tumors, may lead to serious and even fatal loss of blood.

In the three glass test, blood only at the end of urination suggests a source in the bladder, only at the beginning, a source in the urethra, but evenly mixed blood a source in the kidney. If the red cells are imbedded in casts, a renal source is certain. If the red cells are much altered, either crenated or swollen and partly hemolyzed, a renal source is most probable but not certain.

The causes are

(a) *Acute and Subacute Glomerular Nephritis*—Gross hematuria is usually present at some stage and the diagnosis should not be made if red cells are not found. A pure nephrosis, on the other hand, never gives rise to hematuria.

(b) *Focal or Embolic Nephritis*—This is sometimes called hemorrhagic nephritis because of the marked tendency to hematuria.

(c) *Chronic Diffuse Nephritis*—This shows a constant slight hematuria. A marked increase in the number of red cells gives warning

¹ Eisendrath D. N. Hematuria: Its Interpretation. J. A. M. A. 86: 825-828 (March 20) 1926.

Van Duzen R. I. Review of 500 Cases of Hematuria. Texas State J. Med. 23: 57, 180 (Jan.) 1928.

Mackenzie D. W. Hematuria. Canad. Med. Assoc. J. 27: 405-406 1932.

Willur D. I., and Priestly J. T. Essential Hematuria. A Clinical and Pathologic Study. Ann. Surgery 101: 647-659 (Jan.) 1935.

of the onset of one of the acute exacerbations so common in this disease. The presence of red cells is an important differential point between this condition and the primary contracted kidney of hypertensive cardiovascular renal disease in which hematuria is absent.

(d) *Tuberculosis of the Kidney*—Hematuria is present in about 50 per cent of the cases. It may be one to four plus and may occur early or late in the course of the disease. It is usually intermittent but may be constant. Pus cells are usually present also. The bleeding may be from the kidney or from the ulcers in the bladder which are usually present.

(e) *Drugs and Poisons*—A large number may produce this symptom. The most important are mercury, bismuth, arsenic, phosphorus, cantharides, turpentine (paints), methenamine (urotropin), sulfapyridine and insulin. The first five should not be given if renal hematuria is present and should be discontinued if it appears. Hematuria is not necessarily a contraindication to the administration of sulfapyridine, methenamine or insulin but it is usually advisable to decrease their dosage if it appears to result from their administration, as it occasionally does.

(f) *Neoplasms*—All malignant tumors of the kidney may give rise to hematuria, but carcinoma (hypernephroma) is the most important. The bleeding occurs in over 80 per cent of the cases, is usually the first symptom to appear, and is apt to be intermittent at first and later become constant.

(g) *Infarction of the Kidney*—This usually leads to a transient gross hematuria.

(h) *Pyelitis, Pyelonephritis and Pyonephrosis*—These give rise to one to two plus hematuria in the more severe cases, but pyuria dominates the picture.

(i) *Hydronephrosis*—This, even when uncomplicated, may result in intermittent gross hematuria.

(j) *Renal Calculi*—These frequently give rise to gross or microscopic hematuria, but gross hemorrhage into the renal pelvis may produce clots, which in turn may cause typical ureteral colic and lead one to suspect a calculus which is not present.

(k) *Trauma to the kidney*

(l) *Congenital Polycystic Kidneys*—In from 50 to 70 per cent of cases these give rise to intermittent gross hematuria over a period of years.

(m) *Extreme Passive Congestion of the Kidney*—This may give rise to one to two plus hematuria which disappears when the congestion decreases.

(n) *Systemic Diseases with a Hemorrhagic Tendency* (p 273) —The most important are

(1) Purpura hemorrhagica and other conditions in which the platelet count is low These include aplastic anemias, leukemias and a few cases of pernicious anemia

(2) The group of athrombopenic purpuras

(3) Hemophilia

(4) Hodgkin's disease and lymphosarcoma

(5) Polycythemia vera

(6) Scurvy

(7) One form of vicarious menstruation

(8) Periarthritis nodosa

(o) *Essential Hematuria* —This is the term used when no cause can be found The cases so reported are probably merely failures in diagnosis most often of a focal embolic nephritis of low grade, or of a group of miscellaneous minute lesions of the renal pelvis or papillae

Since the terms essential, idiopathic and functional usually mean *cause undetermined*, they should be used with caution

In all the above groups the red cells enter the urinary tract in the kidney or renal pelvis In the remaining types of hematuria the red cells enter the urinary tract distal to the renal pelvis

(p) *Severe Infections* —Microscopic or gross blood is found in the hemorrhagic forms of ureteritis, cystitis, and urethritis, and may also be due to severe infections of the prostate or seminal vesicles Ureteritis with hematuria occasionally results from adjacent inflammations (appendicitis or salpingitis), and focal nephritis may also occur in such conditions

(q) *Calculi* may be formed either in the bladder or in the renal pelvis Hematuria is particularly apt to occur during their passage through the ureter or urethra

(r) *Neoplasms*, either primary or secondary, anywhere in the urinary tract Particularly severe hemorrhages may occur from tumors of the bladder Malignancies of the prostate or of the female genitalia are especially apt to invade the urinary tract and produce hematuria through ulceration or obstruction and resulting infection

(s) *Ulceration of all types* occurring in the urinary tract

(t) *Trauma* including the trauma of instrumentation

(u) *Bilharziasis* is a common cause of hematuria in Egypt and Northern Africa and has been reported in the Southern United States The ova of *Schistosoma hematobium* will also be found in the urine in such cases

A urologic examination by an expert is definitely indicated in any case of persistent or recurrent hematuria in which the history, physical

examination and laboratory results do not clearly indicate the cause

3 **Hemoglobinuria**¹—This is the presence in the urine of hemoglobin unassociated with red cells. While not a sediment this is most conveniently considered at this point because it must be differentiated from hematuria. Albumin and casts are usually associated. It is very much less common than hematuria, from which it must be sharply distinguished. It results whenever extensive hemolysis occurs in the circulating blood. One per cent of the total hemoglobin, or about 8 grams,² must be liberated within a relatively short period of time in order to produce it. Hence, if found anemia should be looked for. Unless enough alkalis are administered to make the urine alkaline, the pigment is precipitated in the tubules resulting in oliguria with impairment of kidney function and even death from uremia. The causes are

(a) *Paroxysmal Hemoglobinuria*³—The hemoglobinuria in this condition comes on after exposure to cold. The mechanism is almost certainly the uniting with the red cells of an auto- and isohemolysin which is present in the blood stream in these cases. This union occurs only at low temperatures. The presence of complement is necessary for hemolysis but not for this union. Attacks may be induced artificially by immersing the upper or lower extremities in cold water. The diagnosis should be confirmed by the Donath Landsteiner test. Most if not all of these patients have syphilis. Hence paroxysmal hemoglobinuria is a definite indication for a Wassermann (positive in 90 per cent of cases) or Kahn test, and for a careful clinical search for evidences of syphilis.

(b) *Blackwater Fever*—Some cases of malaria usually while under quinine therapy, develop an extreme hemoglobinuria from which the name blackwater fever is derived. The mechanism is disputed but the estivo autumnal parasite seems to be responsible for most cases.

(c) *Hemolytic serum reactions*, such as those following the transfusion of incompatible bloods and, in rare instances, introduction of therapeutic serum preparations such as those for diphtheria, tetanus, meningococcus meningitis, etc.

(d) *Poisoning with Hemolytic Drugs or Toxins*—This group includes the following

(1) Bites of most poisonous snakes, toads and spiders

(2) Arsine. This is one of the gases used in the war. It occurs also as an industrial risk and has as its chief toxic effect the production of an extreme hemoglobinuria.

(3) Poisonous toadstools

(4) Several plant toxins, such as ricin, croton, robin, etc. These rarely cause human poisoning. Favism, which is characterized by hemoglobinuria, fever, jaundice, and anemia, is an allergic reaction to the inhalation of the pollen or the ingestion of the bean of a lentil (*Vicia fave*) used chiefly by Italians and Sicilians.

¹Weir J. F. Recurring Acute Hemolytic Crisis with Hemoglobinuria. Proc Staff Meetings Mayo Clinic 8: 110 (Feb 15) 1933.

²Ottenberg R. and Fox C. L. Jr. The Rate of Removal of Hemoglobin from the Circulation and Its Renal Threshold in Human Beings. Am J Physiol 123: 516-525 (Aug) 1938.

³Mackenzie G. M. Paroxysmal Hemoglobinuria. Med 8: 159-193 (May) 1929.

Witts L. J. The Paroxysmal Haemoglobinurias. Lancet 2: 115-120 (July 18) 1936.

(e) *Extensive Superficial Burns*—These may lead to absorption of sufficient split protein to produce hemoglobinuria

(f) *Raynaud's Disease*—This has been reported to be accompanied by intermittent hemoglobinuria in some instances

(g) *Hemolytic anemias*, such as hemolytic icterus Lederer's acute febrile anemia Winckel's epidemic hemoglobinuria of the newborn, pernicious anemia in rare instances etc

(h) *Exanthemata* in the hemorrhagic forms

(i) *Paroxysmal Nocturnal Hemoglobinuria*¹—This is very rare

4 **Methemoglobinuria**—This may result from the administration of chlorates, nitrites, nitrates, phenylhydrazine, or aniline derivatives (especially acetanilid), or, rarely, from nitrite formation by bacterial action in the intestine. Patients who are receiving sulfanilamide or related compounds may become cyanotic, and some² believe this to be due to methemoglobinemia. Others³ believe the color is due to a dye formed by the action of ultraviolet light on sulfanilamide. A rare familial idiopathic form has been described. It may be confused with hemoglobinuria or sulphhemoglobinemia.⁴

5 **Porphyrinuria** (hematoporphyrinuria or hematoporphyrria)⁵ This is an increased rate of excretion of uroporphyrin or coproporphyrin, not of hematoporphyrin as the common name suggests. It occurs as a rare congenital anomaly, in pellagra, and, most commonly, after the ingestion of lead, barbital, sulphonal, trional, tetronal or quinine.

6 **Pyuria**—This is the presence of pus cells or leukocytes in the urine. If inflammation of the genito urinary tract is suspected (smarting, or frequent urination, unexplained fever with chills, or many pus cells in voided urine) and urethritis has been excluded a catheterized specimen should always be examined. All interpretations given below are based on the findings in urine obtained by catheter. A few leukocytes will, of course, be present in all cases of hematuria and their presence in the proportions in which they occur in the blood does not alter the interpretation. Pus, not associated with red cells or in amounts disproportionate to the number of red cells, is significant of

¹ Dacie J V, Israels M C G and Wilkinson J I. Paroxysmal Nocturnal Haemoglobinuria of the Marchafava Type. *Lancet* 1: 479-482 (Feb 26) 1938.

² Hartmann A F, Perley Anne M and Barnett H L. Study of Some of the Physiological Effects of Sulfanilamide. II. Methemoglobin Formation and Its Control. *J Clin Investigation* 17: 699-710 (Nov) 1938.

³ Ottenberg K and Fox C L Jr. Explanation for the Cyanosis of Sulphanilamide Therapy. *Proc Soc Exper Biol & Med* 38: 479-481 (May) 1938.

⁴ Healey J C. Sulphemoglobinemia. *J Lab and Clin Med* 18: 348 (Jan) 1933.

⁵ Mason V R and Farnham R M. Acute Hematoporphyrria. *Arch Int Med* 47: 467 (March) 1931.

Mason V R, Courville C and Ziskind F. Porphyrins in Human Disease. *Medicine* 12: 355-439 (Dec) 1933.

inflammation in the genito urinary tract Its source should be determined by stripping the urethra and using the three glass test Pus is an indication for staining a smear of the sediment of the fresh urine with methylene blue If the methylene blue stain shows bacteria, a Gram's stain is desirable If no bacteria are seen, a specific stain for tubercle bacilli should be made on the centrifugated sediment (pages 507 and 508) If this is also negative, urine or sediment from a urine, collected with aseptic technic, should be inoculated into suitable culture media and into a guinea pig

The causes of pyuria are

(a) *Tuberculosis of the Kidney*—Pyuria with or without hematuria should always arouse suspicion of this condition if cultures and Gram's stain are negative Tubercle bacilli may be discovered in the urine by stain, culture, or guinea pig inoculation after concentration by the technic given

(b) *Other Infections of the Genito urinary Tract*—The most common are urethritis, cystitis, pyelitis, pyelonephritis, pyonephrosis, or some combination of these, but pus may also occur from prostatitis, seminal vesiculitis, or the rupture of an abscess into the urinary tract This latter is suggested if a large amount of pus is suddenly passed Any of the other causes mentioned may give rise to one to four plus pus The amount depends more on the extent and severity of the inflammation than on the type of infection In all cases the causative organism should be demonstrated by stain and culture The gonococcus is the most common cause of urethritis, the colon bacillus, of cystitis and pyelitis, while streptococci and other pyogenic organisms are also frequent etiologic agents

(c) *Trauma*, including that of instrumentation This may produce a slight temporary pyuria

7 *Epithelial Cells*—These occur in most urine specimens and are of little diagnostic value A very large amount of renal epithelium should suggest mercury or bismuth poisoning Epithelium from the vagina must be excluded

8 *Doubly Refracting Lipoid Droplets* (identified best with the aid of the polarizing microscope)—These may be either intracellular or extracellular and are characteristic of lipoid nephrosis They are believed to be composed of cholesterol esters

9 *Fat Droplets* (identified by reddish orange color on staining with Sudan III) These occur in fatty degeneration of the kidney (phosphorus or mercury poisoning), in lipemia (excessive ingestion of fat, diabetes mellitus, alcoholism), or as a contamination from vaseline or oil used as a catheter lubricant

10 *Chyluria*—This occurs chiefly in Egypt, India, Brazil, and Japan. The urine appears milky or, if blood is present also, reddish white. It contains protein and sometimes, sugar as well as fat. Ether extraction partially or completely clarifies it. The droplets are so fine they can be distinctly seen only with darkfield illumination. It results from rupture of a lymph vessel into the urinary tract and is usually due to filariasis. Hence a search for the larvae in the blood is indicated if chyluria is detected.

Addis¹ suggests a method of counting casts, red cells, and leukocytes after a day's abstinence from fluids, which is of value in research, but is a time consuming procedure and is seldom necessary for clinical purposes. An excretion in the 12 hour test period of over 5 000 casts, 500 000 erythrocytes or 1,000 000 leukocytes and epithelial cells is definitely abnormal. The average normal excretion of these elements in the 12 hour period is about 1 000 casts, 70,000 erythrocytes and 300 000 leukocytes and epithelial cells. Casts to 100 000 would have the same significance as 1 plus and over 4 000 000 the same as 4 plus in table 4 (p. 45). Erythrocytes to 100 000,000 would have the same significance as 1 plus, and up to 1,000 000 000 the same as 2 plus. Leukocytes and epithelial cells to 50 000,000 would be interpreted as 1 plus and to 500,000 000 as 2 plus as the terms are used in this book. An excretion of more than 50 000 000 pus cells would favor a diagnosis of pyelonephritis rather than glomerular nephritis.

11 *Crystalline Sediments*—These tell us little with these exceptions.

(a) The presence of *leucin* and *tyrosin* crystals. This indicates serious damage to the liver. They sometimes occur in acute yellow atrophy and phosphorus poisoning both extremely rare conditions. These crystals have been reported in some cases of so called catarrhal jaundice, a further point in favor of the view that this condition is really a diffuse hepatitis. Millon's test will sometimes detect tyrosin in excess even when it does not crystallize.

(b) *Triple phosphates* and *ammonium urate*. Large numbers of these crystals in *freshly voided* urine are found only when it has remained for some time in the bladder and undergone ammoniacal decomposition. This type of decomposition is due to organisms which have the property of hydrolyzing urea to ammonia. The latter not only makes the urine strongly alkaline, thus precipitating the crystals mentioned, but also produces chemical irritation of the bladder wall, often amounting to a severe hemorrhagic cystitis. Hence when one finds such urine one may conclude that the two prerequisites are present, i. e., a cause of stasis of urine in the bladder such as enlarged prostate, stricture or paralysis of the bladder musculature, and infection with an organism capable of producing ammoniacal decomposition.

(c) *Cystin Crystals*²—The presence of cystin or cystin crystals in the urine is a congenital anomaly of metabolism called cystinuria. Demonstration of cystinuria is an indication for alkalization of the urine to prevent the formation of calculi or to dissolve any which may be found.

(d) Other urinary sediments should be recognized but are of little diagnostic significance. A cloud of phosphates may produce a urinary turbidity which alarms the patient but is not pathologic. A heavy sediment of urates or uric

¹Addis T. and Oliver J. *The Renal Lesion in Bright's Disease*. Paul B. Hoeber Inc. New York 1931.

²Lewis H. B. *Cystinuria: A Review of Some Recent Investigations*. Yale J. Biol. and Med. 4: 437 (March) 1937.

acid may give a beginner the impression of gross hematuria but a microscopic study will determine the nature of the sediment

Calcium oxalate crystals are common in normal urine and, when present in large numbers, may cause mechanical irritation of the urethra (oxaluria) but are not diagnostic of calculus formation as is sometimes stated

12 **Calculi**—If these are passed or obtained at operation, they should always be examined as to their chemical composition as this information may make possible a regulation of diet, fluid intake and reaction of urine which will tend to prevent further calculus formation It has been suggested, however, that focal infection is a more important factor in calculus formation than is the composition of the urine Finding calcium phosphate calculi suggests a search for evidence of hyperparathyroidism¹

IV RENAL FUNCTION TESTS

The normal kidney has enormous reserve power as is evidenced by the fact that one kidney² may be completely removed without any demonstrable harm to the individual or impairment of function demonstrable by the tests in clinical use at the present time Hence, a normal result by any of the tests now used does not mean that the kidney is undamaged but impairment of renal function as shown by these tests, even though slight, does mean that temporarily, at least, a very considerable portion of renal substance is not functioning They are of most value in chronic nephritis when the diagnosis is most difficult by other methods

A *The Volume and Specific Gravity* of the 24 hour urine This should always be determined in suspected kidney disease, cardiac decompensation, diabetes mellitus, diabetes insipidus, in any patient with edema or fluid accumulations in the body cavities, and whenever the patient's statement leads one to suspect an alteration in urinary output In most cases, where indicated at all, the record should be kept daily until the volume has returned to normal and remained so for ten days It is of much more value if a record of the fluid intake is kept for the same period Inaccurate collection will vitiate the value of the results, hence, the importance of carefully observing the precautions given in Part II cannot be overemphasized

The determination of the specific gravity is usually included in the routine urinalysis, but practically no information of value is obtained from this determination unless the specimens are collected under conditions of the concentration and dilution test or as accurate 24 hour

¹ Albright F Baird P C Cope O and Bloomberg Esther Studies on the Physiology of the Parathyroid Glands IV Renal Complications of Hyperparathyroidism Am Med Sci 187 49 (Jan) 1934

² Ellis L B and Weiss S The Renal Function in Persons with One Kidney Med Sci 186 242-248 (Aug) 1933

samples In my opinion, this procedure could be eliminated from routine urinalyses and done only when specifically indicated

1 **Normal Values**—The normal urine volume is a function of the fluid intake and of the amount of loss of fluid either by perspiration (visible and invisible) or from the lungs and intestinal tract Hence, it is variable in different climates, in races with different drinking habits, and even in the same individual under different circumstances In the United States, however, 24 hour urine volumes usually fall between 800 and 1800 cc and volumes under 600 cc or over 2000 cc should be investigated

The total solids excreted daily are less variable (for adults about 40 to 60 grams) than are the fluid variations As a result the specific gravity tends to vary in inverse ratio to alterations in volume both in the normal and in most disease conditions The normal range for the specific gravity of 24 hour samples in this country is from about 1.008 to 1.028, with most results between 1.015 and 1.025

Normal Children—More data on the urinary output of children in this country are urgently needed¹ Apparently the variations in specific gravity are similar to those in adults, and the urine total solids for children 1 to 6 years of age are about one fourth and for children 6 to 12, about one half the adult values The urine volume for children 1 to 6 years is 200 to 800 cc and for children 6 to 12 years of age, 400 to 1000 cc

2 **Physiologic Variations**—(a) *Increased perspiration* from any cause This tends to decrease the volume and raise the specific gravity, hence, in spite of large fluid intake, urine volumes tend to be low in persons in the tropics and in those doing hard physical labor

(b) *Decreased Perspiration*—This is most often due to exposure to cold and tends to increase the urine volume and decrease the specific gravity

(c) *Drinking and Eating Habits*—These have a marked influence In those countries in which beer and wine are much used, urine outputs of less than 2000 cc are uncommon Coffee and tea produce a specific diuretic action in addition to the effect of the volume of fluid Total solids will vary with the amount and character of the food eaten and with the endogenous metabolism

3 **Oliguria**—This is a decreased formation of urine which is usually deeply colored and of high specific gravity When the formation is totally suppressed, it is called anuria These must not be con-

¹ The figures given in texts are based on old results of German and Russian studies and are certainly too high for this country

fused with retention of urine, a catheter should always be passed if there is any doubt

The pathologic causes are

(a) *Acute Glomerular Nephritis*—Oliguria may be the first symptom noted by the patient. Occasionally complete anuria may occur. This is one point of some value in differentiating it from focal embolic nephritis in which the urine volume remains normal. As the condition improves or as it goes into the subacute stage the volume and specific gravity return toward normal. If it progresses further to the stage of chronic diffuse nephritis, a polyuria develops.

(b) *Acute exacerbations* of chronic diffuse nephritis—A reduction from the preceding increased volume always occurs and an actual oliguria is usual.

(c) *Eclampsia* and the *uremic stage* of all types of impairment of renal function.

(d) *Poisoning*—Mercury, lead, bismuth, arsenic, turpentine, or cantharides may produce oliguria or anuria.

(e) *Hemoglobinuria and methemoglobinuria*

In the above groups specific diuretics and forcing of fluids are contraindicated as renal ability to eliminate fluid is impaired.

(f) *Nephrosis* and *renal amyloidosis*—These are constantly associated with oliguria during the stage when the edema is developing.

(g) The stage of *development of edema* or *accumulations of fluid* in any of the body cavities—It is the rule in congestive heart failure, severe anemia, pleuritic effusions, or ascites, no matter what the cause.

Groups (f) and (g) constitute specific indications for limitation of fluid intake and the use of diuretics.

(h) *Deficient ingestion or absorption* of fluids from any cause.

(i) *Excessive loss of fluids* from vomiting, diarrhea, excessive perspiration, or severe burns.

(j) *A fall in blood pressure*—This occurs in postoperative, traumatic or hemorrhagic shock, in Addison's disease, and in moribund patients.

Groups (h), (i), and (j) do not call for treatment with diuretics, but constitute specific indications for forcing fluids, subcutaneously or intravenously if necessary to secure absorption.

The oliguria occurring in most *febrile diseases* is due generally to a combination of causes mentioned in groups (h), (i), and (j) and therefore should disappear with the institution of proper therapy.

4 *Polyuria*—This is an excessive formation of urine, usually of pale color and, with the exception of the polyuria of diabetes mellitus, of low specific gravity. It should not be confused with frequency of

urination as either may occur independently, although they often co exist

The important causes of polyuria are

(a) *Chronic Diffuse Nephritis* —In this disease, with the exception of the acute exacerbations and the terminal stage, polyuria is constant although the volume is usually less than 3000 cc per day

(b) *Hypertensive Cardiovascular Renal Disease* —This gives a similar polyuria, most marked at night, as the earliest and most constant evidence of renal involvement

(c) *Diabetes Insipidus* —This is an uncommon condition due to a lesion of the posterior lobe of the pituitary or the adjacent area of the brain, in which the urine volume may reach the astounding values of 3 to 30 liters per day with a specific gravity as low as 1.001. It is differentiated from diabetes mellitus by the fact that the urine does not contain sugar. Desiccated posterior pituitary intranasally¹ causes a return to normal volume

(d) *Diabetes Mellitus* (see Chapter III) —This is the chief exception to the rule of inverse ratio between the volume and specific gravity, as the specific gravity is usually normal or high in spite of the increased volume

(e) During the *disappearance of edema or fluid accumulations* in the body cavities whether this be spontaneous or the result of therapy

In the above groups frequency of urination may or may not occur, but is usually less marked than one would expect from the increased volume

(f) *Reflex polyuria* may result from any irritation in the urinary tract, such as calculi, obstruction, cystitis, pyelitis, tuberculosis, or from nervousness or hysteria, but in all of this group frequency of urination is more constant and greater in proportion than the increased volume

B Ratio of the Night to the Day Volume of Urine —One of the first symptoms noted by patients with chronic nephritis or hypertensive cardiovascular renal disease is that they have to interrupt their sleep to void urine. This ratio should be determined as a quantitative check if increased night volume is suspected. It is better to combine it with the modified Mosenthal test (C)

1 Normals —In adults the normal ratio of the night to day volumes is N D 1 2, 3, or 4. In children this test is of little value as a ratio of N D 1 1 is not uncommon in normals

¹ Vidgoff B. Posterior Pituitary Therapy in Diabetes Insipidus. Endocrinology 16: 289-292 (May-June) 1932

2 Increased night volume giving a ratio of N D 1 15, 10 or even less is constant enough to be of diagnostic value in chronic diffuse nephritis or hypertensive cardiovascular renal disease. Other conditions which may also give this result are mentioned below (see C, 2 and 3)

C *The Modified Mosenthal Test*—This has been largely superseded by the more sensitive concentration, dilution and urea clearance tests. The interpretation given here applies only to tests carried out by the technic given, which permits the calculation of the N D ratio. It is of some value in all patients with hypertension who do not have edema, marked oliguria, or a blood urea nitrogen of over 50 mg per 100 cc.

1 **Normal Findings**—The specific gravity is 1 020 or more in at least one sample with a variation of at least nine points between the highest and lowest specific gravity. The 12 hour sample should measure 400–600 cc, occasionally as high as 750 cc. If nitrogen and sodium chloride are estimated, the concentrations will vary considerably in different samples, and are usually as high as 1 per cent in at least one sample. The total volume and night to day ratio will fall within the ranges given above (B).

In children the interpretation is much the same except that the maximum variation in specific gravity of 8 points may be normal and the total volume and night to day ratio are as given above (A and B).

2 In the chronic forms of impaired function the night volume (12 hour sample) is increased, the specific gravity of the samples is lowered (highest not usually over 1 015) and it is *fixed*, that is, there are less than 9 points variation in specific gravity between the highest and lowest. There is also a fixation in the concentrations of sodium chloride and nitrogen and they do not reach 1 per cent in any sample. This test may, therefore, give one of the earliest clues to chronic impairment of renal function such as occurs, constantly in chronic diffuse nephritis, and at some stage in most cases of hypertensive cardiovascular renal disease, congenital polycystic kidneys, or bilateral hydronephrosis (prostatic hypertrophy), pyelonephritis, or pyonephrosis.

The following conditions, none of which constitutes an indication for doing the test, must, however, be ruled out as they give similar results although renal function is not impaired. They are pyelitis cystitis, diabetes insipidus, severe anemias, and edema during the stage of disappearance.

3 Other conditions which give abnormal results of a different type but do not constitute an indication for doing the test are all the causes of oliguria mentioned. These produce a fixation of the specific gravity at a high level with often an abnormal night to day ratio.

D The Concentration and Dilution Tests¹—These are among the most sensitive tests available for detecting *slight grades* of impairment of renal function of any type. The test is contraindicated in severe grades of renal damage as it puts severe strain on the kidneys, and should not be done if edema is present as unreliable results are secured. Its chief value is in detecting early renal damage in hypertensive cardiovascular renal disease and early chronic diffuse nephritis and in following the course of these conditions up to the stage of definite nitrogen retention. After this stage the estimation of blood urea nitrogen gives more reliable information with less chance of injury to the patient.

1 **Normal Values**—The specific gravity rises to 1.028 to 1.030 in the concentration test and falls to 1.003 or lower in the dilution test. The urine collected in the first four hour period of the dilution test should amount to 1200 to 1800 cc.

2 In the chronic forms of renal damage the first change that occurs is an inability to concentrate while the ability to dilute the urine remains quite good. This is called *hyposthenuria*. As the impairment becomes more severe the ability to dilute is also lost, so that the urine has an almost constant specific gravity, varying between 1.009 and 1.013 only, practically the same specific gravity it would have if it were only a dialysate of the blood plasma. This is called *isosthenuria* and indicates severe renal damage.

3 In acute renal damage ability to dilute is first lost and fixation at a high level results, if the damage becomes more severe an isosthenuria may also result. Since other causes of oliguria may give similar findings it is of less value in acute cases.

L. The Phenolsulphonphthalein Test²—This is indicated in any case in which the preceding tests are positive or when impaired renal function is for any reason suspected.

If the intravenous technic is used in conjunction with ureteral catheterization it may be used to determine the function of one kidney. The normal appearance time of the drug by the intravenous technic is 3 to 5 minutes with a total excretion

¹Lebermann F. Der Wasserversuch als Nierenfunktionsprüfung. Theodor Steinkopff Dresden 1932.

²Chapman F. M. and Halsted J. A. The Fractional Phenolsulphonphthalein Test in Bright's Disease. Am J Med Sci 186: 223 (Aug) 1933.

by each kidney of 15 to 25 per cent in the first 15 minutes, 25 to 30 per cent in the first 30 minutes, and 30 to 40 per cent in the first hour. The totals for the two kidneys should, of course, be twice the above values.

The figures given below are for the mixed bladder urine.

1 Normal Results —These are the same for adults and children

Intramuscular	Intravenous	Per Cent
Excretion during the first hour	First half hour	40-60
Excretion during the second hour	Second half hour	20-25
Total excretion for the two hours	One hour	60-80

2 Pathologic Results

	Per Cent
(a) Slight impairment total for 2 hours	One hour 30-50
(b) Moderate impairment total for 2 hours	One hour 10-30
(c) Severe impairment total for 2 hours	One hour 0-10

There is a tendency for the proportions to change, i.e., proportionately less dye excreted the first hour and more the second hour, as the function decreases. The causes of pathologic results are the same as those given below for elevation of the blood urea nitrogen, but it is slightly more sensitive to mild grades of impaired function and much less reliable for determining the severity of moderate and extreme grades of impairment of renal function. Low excretion, not due to impairment of kidney function, may occur if the test is performed less than 5 hours after the administration of magnesium sulphate. A total excretion of more than 80 per cent suggests impaired liver function, since normally the liver excretes part of the dye into the bile.

3 The chief objections to this method are

- That the substance used for the test is not one normally excreted by the kidneys
- That it tests the renal function only for the two hour period

The methods to be discussed next all depend for their value on the fact that certain substances increase in concentration in the blood, probably due to an inability on the part of the kidney to excrete them when the renal function is impaired. They are all normally present in the blood in definite concentration and are normally excreted by the kidneys. Since many of them occur in food or are products of its digestion and metabolism, all blood samples for blood chemistry work should be taken after a fasting period except in emergencies (e.g., coma, eclampsia, etc.). In the morning before breakfast is satisfactory.

F Blood Urea Nitrogen Estimation —This test should be done whenever impaired function is suspected. The estimation should be

repeated at frequent intervals as a check on the progress of the case when impaired function has been found

Advantages of this method are that urea is a substance normally excreted by the kidneys and the estimation gives an average of the renal function for a period of time. It is the first substance to be retained in the blood when renal function is impaired, and the quantity present gives a good indication of the amount of impairment, while repeated estimations show the response to treatment. Since one sound kidney is effective for normal excretion, it will not detect even severe impairment of only one kidney as will the phenolsulphonphthalein test, nor will it detect as slight grades of impairment of function as will the urea clearance test and the dilution and concentration tests. Because of its simplicity and dependability and the lack of contraindications to its use, it is, in my opinion, the most valuable clinical test of renal function and the one to do first.

1 **Normals**—The figures usually given are 12–15 mg of urea nitrogen¹ per 100 cc of blood, but lower figures (down to 7 mg) are frequently secured. Pregnant women are especially apt to give low estimations (5 to 12 mg). Figures for children are the same as for adults. In apparently healthy men over 40,* values up to 20 mg are reported.

2 Pathologic Results

	Mg Urea Nitrogen per 100 Cc
Mild impairment of renal function	16–20
Moderate impairment	30–50
Severe impairment	60–120

In uremia it may go as high as 400 mg but is sometimes as low as 60–70 mg.

3 **Causes of Impaired Renal Function**—In these conditions elevation of the blood urea nitrogen, decrease in phenolsulphonphthalein excretion and diminished urea clearance may occur.

(a) *Bilateral Diffuse Kidney Disease*—Casts are usually present in the urine in this group.

(1) Acute and subacute glomerular nephritis

(2) Chronic diffuse nephritis

(3) Hypertensive cardiovascular renal disease. Urea nitrogen retention is present only in the cases with considerable renal involve

¹ Multiplying the nitrogen figure by 2.15 converts it to mg urea (some report urea in stead of nitrogen of urea).

*Lewis W. H. Jr. and Alving A. S. Changes with Age in the Renal Function in Adult Men. I. Clearance of Urea. II. Amount of Urea Nitrogen in the Blood. III. Concentrating Ability of the Kidneys. *Am J Physiol* 123: 500–515 (Aug.) 1938.

ment, hence rarely occurs early in the disease and may be absent in some advanced cases

(4) Congenital polycystic kidneys

(5) Pyelonephritis

(6) Pyonephrosis

(7) Poisoning with the heavy metals (mercury, lead, bismuth, etc) and with arsenic

(8) Passive congestion of the kidneys

(b) *Dehydration*¹ such as occurs from the following

(1) Prolonged severe vomiting as in high intestinal obstruction

(2) Gastric or duodenal fistula

(3) Severe diarrheas such as cholera

(4) Severe burns

(5) Sodium chloride loss or restriction

(6) Deficient fluid intake This is common in patients with fever or in coma

It is not certain whether this elevation of the blood urea nitrogen in dehydration is a compensatory measure to maintain the osmotic pressure of the plasma at the normal level after excessive loss of salts, whether it is due to the decreased efficiency of the kidney in the excretion of urea with low urine volume (see Urea Clearance, Section IV), or whether it is due to the increased protein catabolism of starvation. Probably all these factors play a part. The prompt return to normal renal function after restoration of the fluid and salt content of the plasma indicates that there is little real toxic damage to the kidney in these conditions

(c) *A marked fall in blood pressure*

(1) Postoperative, traumatic, or hemorrhagic shock. Anhydremia is a contributory factor in the impaired renal function of this group

(2) Addison's Disease² The elevation of the blood urea nitrogen is roughly proportional to the degree of cortin deficiency. This estimation may be used as an index of the response to therapy with cortin, sodium salts and low potassium diet

(3) Moribund patients This includes some cases of diabetic coma

(d) *Obstruction in the Urinary Tract*—This causes elevation of urea nitrogen only in the severe cases in which the total urinary output is

¹ Jeghers H and Bakst H J The Syndrome of Extrarenal Azotemia *Ann Int Med* 11: 1861-1899 (Apr) 1938

² Greene C H, Rowntree L G, Swingle W W and Piffner J J Metabolic Studies in Addison's Disease The Effect of Treatment with the Cortical Hormone of the Suprarenal Gland *Am J Med Sci* 183: 1 (Jan) 1932

decreased. Therefore, it is absent even in complete obstruction of just one ureter if the other kidney is functioning well. Casts are usually absent from the urine in this type

(1) Prostatic enlargement

(2) Stricture of the urethra or of both ureters as in carcinoma of the uterus

(3) Hemoglobinuria,¹ methemoglobinuria or sulfhemoglobinuria. When large amounts of hemoglobin or its derivatives are released in the blood stream, they are excreted in the urine. If the urine is acid, they precipitate in the tubules of the kidney producing obstruction with resulting oliguria or anuria, impaired renal function, and sometimes death from uremia or acidosis. It is important, therefore, that the urine be kept alkaline if one of these pigments is detected in the urine.

(e) *Severe Liver Disease*—This includes acute yellow atrophy,² diffuse hepatitis, terminal stages of cirrhosis, Weil's disease³ and some cases of obstructive jaundice.⁴ The condition is called bile nephrosis by pathologists, but the clinical picture is entirely different from that of typical nephrosis. The bile salts apparently damage the kidney and there may be precipitation of bile pigment in the tubules also. Jaundice is nearly always present. In acute yellow atrophy the nonprotein nitrogen may be elevated out of proportion to the urea nitrogen because of failure of the amino acids to be deaminized.

G *The Nonprotein Nitrogen Estimation*—Some prefer the nonprotein nitrogen estimation to the blood urea nitrogen, but there are more sources of error, it requires more time, and is not so well adapted to small laboratories as is the titration method of blood urea nitrogen determination. The normal results are 25 to 35 mg per 100 cc of blood. The figures run parallel to the urea estimation although, of course, higher, so that little diagnostic information is gained that can not be more accurately and easily obtained by a urea nitrogen estimation. The undetermined nitrogen, which is the difference between the

¹ DeGowin E. L., Oterhagen H. F. and Andersch Marie. Renal Insufficiency from Blood Transfusion. I. Relation to Urinary Acidity. Arch Int Med 59: 432-444 (Mar) 1937.

DeGowin E. L., Warner E. D. and Randall W. L. Renal Insufficiency from Blood Transfusion. II. Anatomic Changes in Man Compared with Those in Dogs with Experimental Hemoglobinuria. Arch Int Med 61: 609-630 (Apr) 1938.

³ Meyers S. G., Brines O. A. and Juliar B. The Acutely Ill Jaundiced Patient. A Report of Twenty One Instances of Hepatic Icterus Seven of Whom Had High Blood Nitrogen. Am J Digest Dis & Nutrition 2: 346-350 (Aug) 1935.

⁴ Glotzer S. Weil's Disease. Report of a Case in a Fish Worker. J A M A 110: 2143-2145 (June 25) 1938.

⁵ Elsom K. A. Renal Function in Obstructive Jaundice. Arch Int Med 60: 1028 1033 (Dec) 1937.

sum of the nitrogen of urea, creatinine, uric acid, amino acids, etc and the total non protein nitrogen, is said to be especially increased in uremia

H Urea Clearance Test¹—This is now recognized as the most sensitive and accurate test of renal function. It is indicated in all cases suspected of having impaired renal function in which accurate collection of urine samples is possible, but is not necessary if the simpler blood urea nitrogen estimation shows retention of 50 mg or over. It is not practical in children under four years of age. Read pages 382–385

1 Normals—The average maximum urea clearance (Cm) is 75 cc of blood per minute cleared of urea. The average standard clearance (Cs) is 54 cc of blood per minute cleared of urea. Normal values are usually reported as percentage of these figures, anything over 75 per cent being regarded as strictly normal and anything under 50 per cent indicating definitely impaired renal function.

2 Physiologic Variations—The urea clearance during pregnancy is variable, some authors reporting decreased clearance and others increased clearance. Severe exercise will somewhat depress the urea clearance in normal subjects and patients with mild impairment of renal function. Moderate exertion will depress the function still further in patients with severe impairment. Administration of vasoconstricting or vasodilating drugs or other factors influencing the rate of blood flow to the kidney may be expected to influence the urea clearance.

3 Pathologic Results—The causes of decreased urea clearance are the same as those given for an elevated blood urea nitrogen. This test, however, detects slighter grades of impairment, since the blood urea nitrogen is not consistently elevated until the urea clearance is below 20 per cent.

4 Advantages—This test is more sensitive than the blood urea nitrogen or phenolsulphonphthalein tests. It has no contraindications as have the dilution and concentration tests. It is not necessary that the subject be in a fasting state. It has the additional advantage of including the blood urea nitrogen estimation as part of the test.

5 Disadvantages—It involves three urea nitrogen estimation and a complicated calculation. Its accuracy on the accuracy with which the urine samples are time and measure

¹Peters J P and Van Slyke D D Quantitative
335–369 Williams and Wilkins Baltimore, 1911

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Catheterization is often necessary to insure complete emptying of the bladder

I Other Clearance Tests—The clearance of many other substances, including inulin,¹ sucrose and creatinine, have been investigated and are of value only in research. They are not necessary in clinical medicine. The inulin clearance gives the best indication of the rate of glomerular filtration as it is apparently not reabsorbed by the tubules

J Blood "Creatinine" Estimation—This test is desirable if the blood urea nitrogen is found to be above 20 mg. The estimation is chiefly of value in prognosis and as a check on the urea estimation. Behre and Benedict³ report experiments suggesting that actual creatinine is not the substance responsible for the color change in this test. This fact does not detract from the clinical value of the test.

1 Normals—Figures of 1-2.5 mg per 100 cc of blood have usually been given, but Holbrook and Haskins⁴ have shown that the upper limit of normals is 1.6 mg. An estimation above 2.0 mg may be considered as definitely pathologic.

2 Pathologic Results—"Creatinine" is more easily excreted than urea so that no "creatinine" retention occurs until there is marked renal impairment, hence, it is of no value for diagnosis of early nephritis. A curve of "creatinine" retention is roughly parallel to the curve of urea above 25 mg of urea nitrogen. Therefore, this serves as a valuable check on the urea estimation, e.g., a normal figure for urea nitrogen and an estimation of 3 mg of "creatinine" on the same blood would indicate an error in one or the other estimation. It is also true that a high urea with a normal or relatively low "creatinine" estimation generally indicates error. Its chief value was formerly thought to be in prognosis, it being claimed⁵ that a "creatinine" level above 5 mg in a patient with chronic impaired renal function indicated death within a few months. However, since a high "creatinine" in acute impairment of kidney function does not necessarily indicate a bad prognosis, and since prac

¹ Shannon J. A. and Smith H. W. The Excretion of Inulin, Xylose and Urea by Normal and Chlorinized Man. *J Clin Investigation* 14: 393-401 (July) 1935.

² Winkler, A. W. and Parra J. The Measurement of Glomerular Filtration. The Creatinine, Sucrose and Urea Clearances in Subjects with Renal Disease. *J Clin Investigation* 16: 869-877 (Nov.) 1937.

³ Behre J. A. and Benedict S. J. Studies in Creatine and Creatinine Metabolism, *J Biol Chem* 52: 11-33 (May) 1922.

⁴ Behre Jeanette A. and Benedict S. R. On the Presence of Creatinine in Blood. *J Biol Chem* 110: 245-248 (June) 1935.

⁵ Holbrook W. P. and Haskins H. D. Blood Uric Acid in Nephritis. *J Lab and Clin Med* 11: 10-18 (Oct.) 1926.

⁶ Myers V. C. and Killian J. A. The Prognostic Value of the Creatinine of the Blood in Nephritis. *Am J Med Sc* 157: 674-695 (May) 1919.

tically all patients with chronic diffuse nephritis die of the disease within a few months to a few years, its value in prognosis is not as great as was originally thought

Causes of a high blood "creatinine" are the same as those listed for urea retention, but "creatinine" retention occurs only after a considerable rise in the urea nitrogen has occurred

K *Other tests used* in cases of impaired renal function

1 Valuable tests to be discussed in more detail in other chapters

(a) *The Alkali Reserve Estimation*—Acidosis due to retention of normally formed acids, such as acid phosphate (NaH_2PO_4), and to loss of base is frequently present in the advanced stages of the types of disease in which there is nitrogen retention, and if such patients are given alkalis or put on a basic diet an alkalosis frequently develops. *Alkali reserve estimations should be done at frequent intervals on all patients with a blood urea nitrogen of over 20 mg. The diet (p. 75) and alkali intake can then be so regulated as to keep the alkali reserve normal. In my experience this is an extremely valuable, sometimes even a life-saving, point which has not been generally recognized.* Alkalis or acids should never be given to patients with impaired renal function unless the alkali reserve is frequently determined as a check on the dosage. The reaction of the urine is not a safe guide in such cases.

(b) *Hemoglobin and Red Cell Count*—Anemia¹ with normal color, volume and saturation indexes is associated with acute, subacute and chronic glomerular nephritis. This seems to be of definite value in differentiating them from the other types of kidney disease in which the hemoglobin is little, if at all, reduced. Anemia may also occur in focal embolic nephritis and very late in those few cases of hypertensive cardiovascular renal disease (type E) in which kidney function is greatly impaired (urea nitrogen over 50 mg.)

(c) *The Blood Chloride Estimation*²—This estimation is indicated when any of the causes of dehydration listed under blood urea are present. Normal values for chloride expressed as sodium chloride in whole blood are 350 to 550 mg. per 100 cc. and in plasma are 570 to 620 mg. per 100 cc. The higher values for plasma are due to the lower chloride content of the red cells. This must be taken into account in interpreting reports of whole blood chlorides because anemia alone will

¹ Brown G. E. and Roth G. M. The Prognostic Value of Anemia in Chronic Glomerular Nephritis. *J. A. M. A.* 81: 1948-1950 (Dec. 8) 1023.

Osgood F. E., Haskins H. D. and Trotman F. E. The Value of Accurately Determined Color, Volume and Saturation Indexes in Anemia. *J. Lab. and Clin. Med.* 27: 859 (June) 1932.

² Peters J. P. and Van Slyke D. D. Quantitative Clinical Chemistry. Vol. I, pp. 1019-1089. Williams and Wilkins Co. Baltimore, 1931.

lead to a higher proportion of plasma and thus to a higher blood chloride level. On the other hand, the plasma chloride level changes so rapidly (see Chapter III) after blood is withdrawn, unless precautions are taken to prevent loss of carbon dioxide that the whole blood chloride estimation is more reliable than the plasma chloride. Impaired kidney function in itself does not lead to chloride retention.¹ A low blood chloride level may, however, lead to anhydremia and impaired kidney function so that salt restriction in patients with impaired kidney function is not desirable unless necessary for the control of edema.

(d) *Blood Phosphate and Calcium Determinations*—The blood phosphate level is elevated and the blood calcium level is depressed in the more severe grades of impaired renal function, but these tests are not necessary for diagnosis except in the rare condition known as renal rickets.² This disease, simulating rickets, develops in patients who survive many years with impairment of renal function sufficient to produce an elevated phosphate and depressed calcium level and to interfere with ossification.

(e) *Protein Content of Effusions*—If ascitic or pleural fluid develops, as is common in nephrosis and in some cases of acute glomerular nephritis, the fluid should be examined. A protein content of less than 10 gm per liter strongly favors a diagnosis of true nephrosis. In glomerular nephritis the fluid usually contains 20 to 30 gm per liter.

2 Tests Which Are Only Occasionally Indicated

(a) *Plasma or Serum Proteins and the Albumin-Globulin Ratio*³—This is part of the thorough study of edema or of nephrosis, but is rarely necessary to establish the diagnosis. It is vitally important, however, to understand the changes in plasma proteins which frequently occur in disease in order to plan proper therapy. A low serum protein may result not only in edema but also in impaired healing of wounds, in rouleaux formation of the red cells and in pseudo agglutination. Alterations in serum proteins are chiefly responsible for the changes in the sedimentation rate. In general the lower the albumin and the higher the globulin and fibrinogen the more rapid is the sedimentation.

¹ Holbrook W. P. and Haskins H. D. Blood Uric Acid. *J. Lab. and Clin. Med.* 11: 3, 7 (Jan.) 1926.

² See reference 1, page 34.

³ Landis J. M., Elsom K. A., Bott I. A. and Shiels I. Observations on Sodium Chloride Restriction and Urea Clearance in Renal Insufficiency. *J. Clin. Investigation* 14: 525-541 (Sept.) 1935.

⁴ Editorial: Renal Rickets. *J. A. M. A.* 111: 256 (July 16) 1938.

⁵ Peters J. I. and Van Slyke D. D. *Quantitative Clinical Chemistry*. Vol. I, pp. 653-24. Williams and Wilkins Co. Baltimore, 1931.

⁶ Hand H. H. Concentration of Serum Protein in Different Types of Edema. *Arch. Int. Med.* 54: 213 (Aug.) 1934.

rate A sedimentation rate of zero or over 100 mm in 15 minutes, therefore, constitutes an indication for a plasma protein determination

The chief factors affecting the level of the plasma proteins are nephrosis, liver disease, a deficient protein intake, an excessive loss of protein in the urine or into transudates or exudates, anhydremia, and, rarely, the presence of Bence-Jones protein in the blood Edema is usually present when the total protein falls below 5.5 gm per 100 cc or the albumin fraction falls below 2.5 gm per 100 cc The specific gravity¹ of the plasma offers a simple means of determining whether the plasma proteins are below this level When the specific gravity is below 1.0235, edema is usually present

(1) *Normals* ²—Normal values for fibrin (spontaneously coagulable fraction, "fibrinogen") are 0.20 to 0.40 gm per 100 cc of plasma, or 3 to 6 per cent of the total protein, for globulin (fraction precipitated by half saturation with ammonium sulphate), 1.0 to 3.0 gm per 100 cc, or 15 to 35 per cent of the total protein, and for albumin, 4.0 to 5.8 gm per 100 cc of plasma or 60 to 80 per cent of the total protein The total protein varies normally from 6.0 to 8.0 gm per 100 cc The albumin globulin ratio varies from 2.0 to 5.0 Figures usually given as normal in most texts differ from these but are apparently based on studies of an inadequate number of questionable normals

(2) *Pathologic Results* —Hyperproteinemia ³—The total protein is over 8 gm, the globulin fraction is increased above 3 gm, and the albumin fraction is usually under 4 gm This change is associated with an extremely rapid sedimentation rate and often with grossly visible pseudo agglutination of the red corpuscles Hyperproteinemia occurs in anhydremia, in multiple myeloma, in lymphogranuloma inguinale, in the sarcoid of Boeck, in leprosy, in kala-azar, and schistosomiasis In multiple myeloma the fraction determined as globulin is sometimes, but not usually, Bence Jones protein A precipitate in serum heated to 56° C, as for inactivation of complement in the Wassermann test, suggests the presence of Bence-Jones protein The albumin globulin ratio is often reversed, that is below one, in this group

Hypoproteinemia —The total protein is less than 6 gm per 100 cc the albumin fraction is very low, the globulin and fibrinogen fractions

¹ Page, I. H. and Van Slyke, D. D. A Simple Test for Plasma Protein Contents Below the Edema Producing Level. J. A. M. A. 99: 1344 (Oct. 15) 1932

² These figures are based on a study of about 80 normals and many pathologic bloods made in my laboratory by the method given in Part II

Harris, L. and Osgood, S. B. Plasma Proteins in Health and Disease. Comparison with the Sedimentation Rate. To be published

³ Jeghers, H. and Selesnick, S. Hyperproteinemia. Its Significance. Internat. Clin. 3: 248-279 (Sept.) 1937

may be normal or increased, the albumin globulin ratio is always reversed, and the sedimentation rate is extremely rapid. Increased fibrinogen is called hyperinosis. Hypoproteinemia occurs in its most marked degree in true nephrosis. It may occur also in nutritional edema from protein starvation, in glomerular nephritis when the loss of protein in the urine has not been replaced by adequate protein intake, in amyloidosis, and in severe liver disease.¹ Hypoproteinemia may occur also in patients with peptic ulcer or other diseases of the gastrointestinal tract who have been placed on a diet too low in protein or who have lost much blood protein through hemorrhage. An operation on such patients before the protein deficiency has been corrected may lead to poor wound healing.²

A reversal of the albumin globulin ratio with increased fibrinogen but normal total protein — In this group the chief deviation from normal is an albumin under 4 gm per 100 cc. The globulin and fibrinogen fractions tend to be increased. This picture may occur in the early stages of all of the conditions listed above as causing hyperproteinemia or hypoproteinemia and, in addition, in all the conditions given as causes of an increased sedimentation rate, the most important of which are infections, malignant tumors, and pregnancy.

Hypoinosis — This is marked diminution or complete absence of fibrinogen with normal total protein and relative proportions of albumin-globulin. This occurs as an extremely rare congenital anomaly (see Hemorrhagic Diseases in the indexes), and in extensive diffuse liver damage (acute yellow atrophy, phosphorus or chloroform poisoning). In this group the sedimentation rate is often low or zero. In severe liver disease there may be low albumin favoring rapid sedimentation rate, or low fibrinogen favoring slow sedimentation rate.

(b) *Uric Acid Estimation* — This method was at one time thought to be the most valuable of any in the diagnosis of early chronic nephritis because it was claimed that uric acid was the first nitrogenous substance to increase in the blood in renal disease. Holbrook and Haskins³ have proved that these claims are false. Of 87 nephritics tested not one of 26 cases showing urea retention of 16-24 mg had any uric acid retention and many with much higher urea figures also failed to show increase of uric acid in the blood. Of the total 87 cases, 80 per cent showed urea

¹ Tumen H. and Bockus H. L. The Clinical Significance of Serum Proteins in Hepatic Diseases Compared with Other Liver Function Tests. *Am J M Sc* 193 788-800 (June) 1937.

² Thompson W. D. Ravdin I. S. and Frank I. I. Effect of Hypoproteinemia on Wound Disruption. *Arch Surg* 36 300-308 (Mar) 1938.

Thompson W. D. Ravdin I. S. Rhoads J. E. and Frank I. L. Use of Lyophile Plasma in Correction of Hypoproteinemia and Prevention of Wound Disruption. *Arch Surg* 36 509-518 (Mar) 1938.

³ Holbrook W. P. and Haskins H. D. Blood Uric Acid. *J Lab and Clin Med* 11 377 (Jan) 1926.

retention, 60 per cent, "creatinine" retention, and only 30 per cent showed uric acid retention. In this 30 per cent there was very little correlation between the amount of uric acid retained and the clinical condition of the patient. The estimation may prove of some value in other conditions than nephritis, such as gout. I have discarded this test entirely in nephritis.

(1) *Normals*—These are usually given as 1-4 mg per 100 cc of blood. Holbrook and Haskins¹ found variations of 1.5 to 3.7 mg in 55 normal individuals, with an average of 2.4 mg.

(2) *Pathologic Results*—Anything over 4 mg may be considered definitely abnormal.

Causes of uric acid retention are so numerous that it is doubtful if the test is of any diagnostic value except in gout. They are

(a) Conditions in which there is increased production from destroyed nuclei such as in leukemias, resolving pneumonia, tuberculosis, etc.

(b) Conditions in which there is deficient excretion such as nephritis, gout, eclampsia.

(c) Starvation, pernicious vomiting, and other causes of ketosis.

(d) Skin diseases especially eczema.

(e) Severe anemias such as pernicious anemia.

(f) Poisons of various kinds (carbon monoxide, wood alcohol).

(g) Cholecystitis.

(c) *The Congo Red Test*²—This is of value in the diagnosis of amyloidosis and nephrosis.

(1) *Normals*—Less than 40 per cent of the dye disappears from the blood plasma in one hour.

(2) *Pathologic Results*—A disappearance of over 60 per cent occurs only in amyloidosis, and is due to adsorption of the dye by amyloid deposits. A disappearance of 40-60 per cent may be due either to amyloidosis or to a true nephrosis. The appearance of visible dye in the urine favors a diagnosis of nephrosis. Since small amounts of amyloid may not decrease the amount of dye by more than 40 per cent in one hour, a normal result does not exclude amyloidosis.

(d) *The Blood Cholesterol Estimation*—This may be done as part of the complete study of a case of nephrosis, thyroid disease, or diabetes mellitus, but these diagnoses are usually readily established by simpler procedures. Normal values range from 100 to 230 mg per 100 cc of blood. High values up to 500 mg are found in nephrosis constantly and in many cases of diabetes mellitus, but as they may also occur in hypothyroidism, pregnancy, nephritis, tuberculosis, cholelithiasis, and numerous other conditions, their differential diagnostic value is not great. Very high results up to 3600 mg, have been reported in diabetic lipemia. A high estimation is an unfavorable prognostic sign in diabetes mellitus.³ The lesions of xanthomatosis contain a great excess of cholesterol, although the blood cholesterol is not usually high in this condition.⁴

¹Holbrook, W. P. and Haskins, H. D. Blood Uric Acid. *J. Lab. and Clin. Med.* 11: 377 (Jan.) 1926.

²Barker, N. W. and Snell, A. M. The Congo Red Test with Special Reference to Excretion of the Dye in the Urine. *J. Lab. and Clin. Med.* 26: 262 (Dec.) 1930.

Lapstein, S. An Evaluation of the Congo Red Test for Amyloidosis. A Correlation of the Autopsy Findings and Dye Absorption in 125 Cases. *Am. J. M. Sc.* 195: 205-211 (Feb.) 1938.

³Rabinowitch, I. M. The Cholesterol Content of the Blood Plasma in Diabetes Mellitus. *Arch. Int. Med.* 43: 363-375 (March) 1929.

⁴Lichty, D. E. Lipoids and Lipoid Diseases. II. Xanthomatosis (Schüller-Christian's

It is of interest that low values for blood cholesterol are the rule in pernicious anemia and hyperthyroidism

Much literature has accumulated on the value of this test in the diagnosis of thyroid and liver disease and nephrosis. In my opinion, the range of variation in normals and in these diseases overlap so much that the test is rarely of enough value in clinical diagnosis to justify the expense for the patient.

(c) *Other Renal Function Tests*—The determination of inorganic sulphate in the serum¹ of the urea indexes of McLean or Addis,² of the urea concentration in the blood after orally administered urea,³ of the ratio of the non protein nitrogen to the urea nitrogen of the salivary urea index of the level of indican in the blood⁴ and many others have been found of value in estimating kidney function. The blood urea nitrogen, the urea clearance test, the phenolsulphonphthalein test, and the concentration and dilution test, however, have proved sufficient for practical clinical purposes.

V SUMMARY OF BLOOD CHEMISTRY NORMALS

TABLE 2.—BLOOD CHEMISTRY NORMALS

	Figures usually given	Figures more probably correct
In Whole Blood		
Urea nitrogen	12-15 mg per 100 cc	7-15 mg per 100 cc
Creatinine	1-2.5 mg per 100 cc	1-1.6 mg per 100 cc
Uric acid	1-4 mg per 100 cc	
Non protein N	25-35 mg per 100 cc	
Sodium chloride	450-500 mg per 100 cc	350-550 mg per 100 cc
Dextrose ¹	80-120 mg per 100 cc	
True dextrose	60-100 mg per 100 cc	
Cholesterol	100-230 mg per 100 cc	
In Plasma		
Alkali reserve	50-80 cc of CO ₂ per 100 cc	
Sodium chloride	570-620 mg per 100 cc	
Total protein	6.0-8.0 gm per 100 cc	
Albumin	4.0-5.8 gm per 100 cc	
Globulin	1.0-3.0 gm per 100 cc	
Fibrin	0-0.4 gm per 100 cc	
Albumin globulin ratio	2.0-5.0	
Icterus index	4-6	
In Serum		
Calcium	9-12 mg per 100 cc	
Icterus index	4-6	

Type) Arch Int Med 54:379 (March) 1934

Cowie D M and Magee M. Catherine Lipoids and Lipoid Diseases. III. Lipoid Content of Tissues in Schüller Christian's Disease (Xanthomatosis) and Review of Literature on Lipoid Content of Human Tissues. Arch Int Med 53:391 (March) 1934

¹ Hoffman W S and Mansfield J V. The Significance of Serum Inorganic Sulphate Concentrations in Bright's Disease. J Lab & Clin Med 21:380-390 (Jan) 1936

² See footnote 1, page 25

³ King E S. The Urea Tolerance Test. Arch Int Med 42:877-892 (Dec.) 1928

⁴ Polayes S H and Eckert Elizabeth Ann. Observations on the Indican Test on the Blood and Urine in Renal Insufficiency. J Lab & Clin Med 20:681-688 (Apr) 1935

VI GRADES OF IMPAIRMENT OF RENAL FUNCTION

It is important not only to detect the presence of renal impairment, but also to know its degree, especially in chronic cases

TABLE 3—GRADES OF IMPAIRMENT OF RENAL FUNCTION

	Urea clearance %	Urea N mg	"Creati- nine" mg	Phthalein (2 hrs)
Slight ¹	20 to 50	7 to 15	1.0 to 1.6	60 to 80 per cent
Mild	15 to 20	16 to 30	1.0 to 2.0	30 to 50 per cent
Moderate	10 to 15	30 to 60	2.0 to 3.5	10 to 30 per cent
Severe	5 to 10	60 to 120	3.5 to 5.0	Trace to 10 per cent
Uremia	Less than 5	120 to 400	5.0 or over	0 to trace

¹ These terms are simply comparative among the grades of impairment detectable by chemical means. Extensive diffuse renal damage is of course necessary to produce even the mildest detectable change. Still milder grades of renal damage will show normal values by these tests but will show changes by the dilution and concentration test.

VII CLASSIFICATION AND DIFFERENTIAL DIAGNOSIS OF THE GROUP OF DISEASES FORMERLY CALLED NEPHRITIS OR BRIGHT'S DISEASE

The accompanying table gives a working classification and differential diagnosis of Bright's disease. This classification was devised by the author and W. P. Holbrook in 1925. Since that time it has been tested by thorough study of several hundred cases in this clinic and others. Using these criteria, medical students soon learn to predict the clinical course and the pathology to be found at necropsy in a high percentage of cases. Our indebtedness to Volhard and Fahr, Bell and Clawson, Kollert, Mosenthal, Christian, Addis, Keith, Epstein, and other distinguished students of the subject is obvious. See table 4.

Letters have been used to designate the different types for two reasons: first, because in the literature the names have often been used so loosely that they are meaningless, and second, because some of these names suggest a knowledge of the pathology of these diseases that we actually do not have. Even the term nephritis is probably not correctly applicable to all of these groups, for it implies an inflammation of the kidney which has certainly not been proved to be present in types A and E. Under the heading of synonyms have been grouped the names used in the literature for the syndrome. The preferred names have been indicated by capitals. The term interstitial nephritis was formerly applied to most cases of Type D and those cases of Type E which showed impairment of renal function. It does not appear in table 4 because it is now obsolete except for a rare form of acute nephritis associated with

TABLE 4—CLASSIFICATION AND DIFFERENTIAL DIAGNOSIS OF BRIGHT'S DISEASE

Type	A	B	C	D	E
Synonyms	NEPHROSIS Tubular or parenchymatous nephritis Degenerative Bright's disease Nephritis with edema	GLOMERULAR NEPHRITIS Hemorrhagic nephritis Nephritis with or without edema	FOCAL NEPHRITIS Hemorrhagic nephritis Embolic nephritis	DIFFUSE NEPHRITIS Glomerular disease Chronic nephritis with or without edema	HYPERTENSIVE CAROTIDOVASCULAR RENAL DYS. Essential hypertension Hyperpiesia Cardio-renal disease Nephrosclerosis Arterio-sclerotic kidney Benign or malignant hypertension Chronic
Course	Acute subacute chronic Under 35 Normal 2-4+ Absent	Acute or subacute Under 40 1-3+ 1-3+ - to 3+	Subacute Under 40 Normal Absent	Chronic Under 50 2-4+ 1+ or absent 1+ to 4+	40 to 65 2-4+ Absent - to 4+
Age	Decreased High 2-4+ 1-3+ Absent Normal None Absent or slight Never	Decreased or normal High or normal 2-4+ 2-4+ 1-3+ Decreased 1-4+ 2-4+	Normal Normal 1-3+ 1-3+ 1-4+ Normal None Absent or slight Never	Increased Low and fixed 1-3+ 1-2+ 1-3+ Decreased 1-4+ 2-4+	Increased Low may be fixed 1+ or - 1+ or - Absent Normal or decreased None to 4+ Absent or slight 8 per cent of cases About 65 per cent Unknown renal ischemia?
Specific gravity	Toxin of pneumococcus or staph (?) Large white kidney with tubular degeneration	Toxin of strept (?) Large red kidney with diffuse inflammation of glomeruli	Bacterial emboli from focus of infection Patchy inflammation or infarction in kidneys	Common Most cases Toxin of strep (?) Secondary contracted kidney	Primary contracted kidney with arteriosclerosis
Albumin	Bl cholesterol high serum and edema fluid proteins low	Bl cholesterol high serum and edema fluid proteins low	Bl cholesterol high serum and edema fluid proteins low	Bl cholesterol high serum and edema fluid proteins low	Bl cholesterol high serum and edema fluid proteins low
Casts					
Red cells					
Proteinuria					
Impaired renal function					
Relative frequency					
Pathology					
Remarks					

scarlet fever The poor term nephrosis is retained because no better term has yet been proposed Nephrosis may prove not to be a disease of the kidney If the physiologists are right, and good evidence supports their views, a degeneration of renal tubules, which the term nephrosis implies, should result in the formation of a very large volume of urine of low specific gravity containing dextrose Actually an almost opposite result occurs

A *Nephrosis*¹ (Type A) —This is a rare disease of young individuals characterized by diffuse edema, hypoproteinemia, reversal of the albumin-globulin ratio, albuminuria, and casts Some² believe that nephrosis is merely a form of acute glomerular nephritis (Type B) in which glomerular permeability to protein is increased without sufficient damage to result in impaired renal function Others,³ with whom I agree, believe there is a separate entity to which the term nephrosis should be limited in which the etiology is probably a disturbance of the formation of the plasma proteins and not a primary disease of the kidney It is differentiated from acute and subacute glomerular nephritis by the absence of anemia, hematuria, hypertension, and impaired renal function It corresponds to the "lipoid nephrosis" of Epstein, but does not include the febrile albuminurias associated with acute infections (pathology parenchymatous degeneration or cloudy swelling), nor the kidney of amyloid disease, nor poisoning with the heavy metals, nor the kidney of obstructive jaundice, all of which are classed as nephroses by many pathologists These conditions should be recognized and diagnosed as distinct entities

B *Acute and Subacute Glomerular Nephritis*⁴ (Type B) —This is differentiated from nephrosis by the presence of hypertension, impaired renal function, anemia, and hematuria It is the common acute or subacute glomerular nephritis associated with hemolytic streptococcus infections such as scarlet fever, sinusitis, and tonsillitis Most of these patients recover completely, a few die in uremia, and a few go on to the chronic stage (Type D) Oliguria is present in the acute stage, but the urine volume increases and the specific gravity decreases as it passes into the subacute stage Glomerular nephritis

¹ Leiter L Nephrosis *Medicine* 10 135-242 (May) 1931

Shapiro P F Lipoid Nephrosis Pathology Genesis and Relation to Amyloidosis *Arch Int Med* 46 137-160 (July) 1930

² Fahr, G What is Lipemic Nephrosis? *Am J M Sc* 194 449-463 (Oct) 1937

³ Murphy F D Warfield L M Grill J and Annis E R Lipoid Nephrosis Study of Nine Patients with Special Reference to Those Observed over a Long Period *Arch Int Med* 62 355-376 (Sept) 1938

⁴ Murphy, F D and Rastetter J W Acute Glomerulonephritis with Special Reference to the Course and Prognosis Study of 150 Cases *J A M A* 111 668-674 (Aug 20) 1938

must be differentiated from poisoning with the heavy metals by the history and physical findings since the laboratory findings are identical. It is differentiated from focal embolic nephritis by the presence of hypertension and impaired renal function.

Those cases of glomerular nephritis with diffuse edema as the most prominent symptom may at times be difficult to differentiate from nephrosis. The presence of any one of the following favors a diagnosis of true glomerular nephritis: hypertension, anemia, hematuria, or impaired renal function. Sometimes the two syndromes may be indistinguishable except at necropsy. This syndrome is spoken of as acute glomerular nephritis with nephrotic syndrome. It results from deficient protein intake to compensate for the protein lost in the urine.

C Focal Embolic Nephritis (Type C)—This is characterized by the presence of albumin, casts and red blood cells in the urine without alterations in volume or specific gravity. It is differentiated from acute or subacute glomerular nephritis by the absence of hypertension, impaired renal function, and diffuse edema. The patchy lesions in the kidney do not destroy enough renal tissue to encroach seriously on the enormous reserve that nature has provided, but are sufficient to give rise to albumin, casts and red cells in the urine. If the micro organism present happens to produce a soluble toxin involving all the glomeruli (which not infrequently occurs), the clinical picture of chronic diffuse nephritis results and at post mortem a kidney showing the lesions characteristic of both focal embolic nephritis and chronic diffuse nephritis is found. This combination must always be suspected when the clinical picture of chronic diffuse nephritis occurs in subacute bacterial endocarditis.¹

D Chronic Diffuse (Glomerular) Nephritis (Type D)—This is occasionally a sequel to acute or subacute glomerular nephritis but more often develops gradually without any acute phase. Acute and subacute exacerbations frequently occur in this type with a temporary return to a laboratory picture approaching that of acute glomerular nephritis. This is characterized by albuminuria, hematuria, impaired renal function, anemia, and hypertension with or without edema. It is differentiated from acute or subacute glomerular nephritis by the longer history, the polyuria, and by the cardiac enlargement indicating a prolonged hypertension. It is differentiated from those cases of hypertensive cardiovascular disease with impaired renal function by the

¹ Bell, I. T. Glomerular Lesions Associated with Endocarditis. *Am J Path* 8: 639-664 (Nov.) 1932.

presence of hematuria and anemia All patients with this disease die within a few years, usually with true uremia

E Hypertensive Cardiovascular Disease¹ (Type E)—This is the form giving rise to the greatest confusion Patients with this condition seldom consult a physician before they are 40 years of age, but often, if routinely examined, they show marked hypertension much earlier (at this stage the condition is often called essential hypertension or hyperpiesis) Next, they develop a night polyuria and, still later, definite evidence of cardiac, renal and cerebral damage In about 60 per cent of these patients cardiac involvement is the major cause of death, the next most frequent cause of death is cerebral hemorrhage or thrombosis, while in only 20 per cent is renal function severely impaired, and in only 8 per cent is uremia the chief cause of death Yet, at some time in almost every case, evidence of some involvement of all three systems may be found The rarity of anemia, the slower progression, the greater age of the patient, and the absence of red cells in the urine are the chief points which aid in differentiating it from chronic diffuse nephritis In the cases which develop marked nitrogen retention, "cottonwool" exudates and edema of the disk are added to the changes in the retinal vessels The terms malignant and benign hypertension may be misleading unless it is remembered that they are merely subdivisions of this one disease Since at least two sets of entirely different criteria (Keith and Wagener, Volhard and Fahr) for the "malignant" group have already been proposed, since many cases starting as the benign form later become malignant, since Goldblatt has shown that either form can be produced experimentally according to the degree of renal ischemia, and since most other disease processes could similarly be divided from a prognostic standpoint into benign and malignant groups it seems wiser to the author to discard the terms benign and malignant in this disease and to evaluate the prognosis of each case individually, making full use, however, of the undoubtedly valuable prognostic criteria proposed for the malignant group The diffuse arteriolosclerosis with uniformly contracted kidneys characteristic of hypertensive cardiovascular disease must be differentiated from the patchy lesions of senile atherosclerosis which result in a nodular kidney without producing hypertension or evidence of impaired renal function, for both conditions lead to the appearance of urinary casts and 1 to 3 plus albuminuria in the urine Confusion has been caused by the discussion in the literature of both the atherosclerotic kidney and that of

¹ Scott R W Hypertension a Century after Bright J A M A 111 2460-2463 (Dec 31) 1938

hypertensive cardiovascular disease under the one designation of arteriosclerotic kidney

Hypertensive cardiovascular disease must also be differentiated from basophil adenoma of the pituitary, adrenal tumors, and coarctation of the aorta which may also give hypertension with slight albuminuria without impairment of kidney function. The differential diagnosis must be made by the history and physical findings

VIII DIFFERENTIAL DIAGNOSIS OF DISEASES¹ WHICH MAY BE CONFUSED WITH THE PRECEDING GROUP

A Congenital Polycystic Kidneys—This condition is rare. The findings are similar to those of chronic diffuse nephritis, but the case runs a longer course with a tendency to occasional gross hemorrhages. Nodular bilateral tumors may often be palpated in the kidney region, and pyelograms show a characteristic shape of the kidney pelvis.

B Hydronephrosis—Impaired kidney function is not detectable except by ureteral catheterization unless the obstruction is bilateral. Strictures of the urethra, prostatic hypertrophy, carcinoma of the uterus, and paralysis of the bladder are among the more common causes. A complete history and urologic and physical examinations are necessary to establish the diagnosis. Hypertension may occur so it must be differentiated from both chronic glomerular nephritis and hypertensive cardiovascular disease. The absence of casts together with a "creatinine" that is low in proportion to the blood urea nitrogen, are points in favor of this diagnosis. Operation should be delayed until renal function is improved to such an extent that the blood urea nitrogen is less than 30 mg. Pyonephrosis is an occasional complication and adds the findings of pain over the kidneys, fever and chills, and pus in clumps in the urine.

C Cystitis and Pyelitis²—Pus, bacteria, mucus, and occasionally blood and albumin may be found in the urine. No impairment of renal function occurs. The urine is sometimes alkaline. The causative organism should be identified by stain and culture of the sediment from urine collected with aseptic precautions. The pH of properly collected 24 hour specimens of urine should be tested if mandelic acid or

¹ Scholl A. J. Urologic Conditions Simulating Chronic Glomerulonephritis. J. A. M. A. 111: 1421-1427 (Oct. 15) 1938.

Urquhart R. W. I. and McCollum J. L. The Urea Clearance Test Compared with Other Renal Function Tests in Urology. Canad. M. A. J. 33: 251-257 (Sept.) 1935.

² Helmholz H. F. and Osterberg A. E. Rate of Excretion and Bactericidal Power of Mandelic Acid in the Urine. J. A. M. A. 107: 1794-1796 (Nov. 28) 1936.

Helmholz H. F. Urinary Infections in Infancy and Childhood. Diagnosis and Treatment. J. A. M. A. 111: 1719-1722 (Nov. 5) 1938.

alkalies are used in therapy If sulfanilamide is used in treatment, ideally, quantitative determination of the sulfanilamide level should be made to be sure that the concentration of the drug in the urine is above 50 mg per 100 cc

D Pyelonephritis¹—This gives the findings of a pyelitis with evidences of impaired renal function in addition It frequently results in true uremia, but the fever and chills, and the finding of a cause, such as obstruction in the urinary tract, differentiate it from chronic diffuse nephritis It is a common cause of death in patients with prostatic hypertrophy, carcinoma of the uterus, tabes dorsalis, multiple sclerosis or other causes of obstruction in the urinary tract or paralysis of the bladder Some cases of chronic pyelonephritis may be confused with hypertensive cardiovascular disease, but the presence of pyuria and anemia and positive cultures on urine obtained by catheter with aseptic precautions should make the diagnosis

E Tuberculosis of the Kidney—Hematuria and pyuria are present with impaired function of the involved kidney in the more severe cases Tubercle bacilli may often be demonstrated by the Ziehl-Neelsen stain on a smear made by the concentration technic, by culture or by guinea pig inoculation with this sediment

F Tumors of the Kidney—Hematuria is usually present Function is not impaired Pyelograms are indicated

G Anhydremia—This occurs particularly in prolonged severe vomiting (high intestinal obstruction, pernicious vomiting of pregnancy, etc) gastric or duodenal fistula, severe burns, profuse perspiration, or severe diarrhea (cholera, etc) The syndrome consists of a low blood chloride level due to loss of hydrochloric acid from the stomach or chloride from the bowel or skin, with alterations in the alkali reserve figure and, in the more severe cases, a retention of nitrogenous substances in the blood Oliguria and albuminuria are usually present, also The alkali reserve figure is usually high, due to loss of hydrochloric acid, but may be brought to normal or lower by a ketosis due to lack of carbohydrate absorption

H Eclampsia—This occurs in the last months of pregnancy, during labor, or in the puerperium It is characterized by hypertension, coma, convulsions, oliguria, 3+ to 4+ albuminuria, 3+ to 4+ casts and usually, also, hematuria Little if any nitrogen retention occurs Ammonia is much increased in the urine The low blood urea

¹Longcope, W T Chronic Bilateral Pyelonephritis Its Origin and Its Association with Hypertension Ann Int Med 11 149-163 (July) 1937

nitrogen (below 30 mg) and the normal "creatinine" estimation differentiate it from uremia

I Amyloidosis of the Kidney¹—This gives rise to laboratory and clinical findings similar to those of nephrosis, but in addition, the congo red test is positive, the liver and spleen are usually enlarged and one is able, as a rule, to find the cause for the amyloidosis such as tuberculosis, osteomyelitis, or chronic infection. In the terminal stages of some cases of amyloidosis, moderate impaired renal function occurs without hypertension

J Poisoning—Mercury, lead² or bismuth poisoning results in oliguria or anuria, hypertension and impaired kidney function with albumin, casts, blood and epithelium in such urine as is voided. The picture is differentiated from acute glomerular nephritis by the history of taking the drug, the presence of a stomatitis often with a dark line near the gums, and chemical tests for these poisons in the stomach contents or urine. Severe gastroenteritis with diarrhea and blood and mucus in the stools is a usual accompaniment

Arsenical poisoning may result in a similar picture or may give rise to exfoliative dermatitis, agranulocytosis, aplastic anemia, purpura hemorrhagica, polyneuritis, or hemorrhagic encephalitis. The particular form of arsenical poisoning depends on individual idiosyncrasy more than on the dose. It must be recognized by the history and by tests for arsenic in the urine

Cantharides poisoning is rare, but results in gross hematuria with oliguria and, often, anuria. There is frequency and urinary tenesmus. Impaired kidney function is the rule

Gastric lavage, administration of specific antidotes, forcing of fluids while preventing cerebral edema by use of a back rest and hypertonic solution, and maintaining the alkali reserve within normal limits will often lead to recovery in these conditions, even though complete anuria lasts many days

K Hemoglobinuria, Methemoglobinuria, and Sulphemoglobinuria—If the urine is not kept alkaline, hemoglobin or its derivatives precipitate in the tubules, producing oliguria or anuria with hypertension, impaired renal function, and often, death in uremia. The

¹Shapiro P F. Lipoid Nephrosis. Pathology, Genesis and Relation to Amyloidosis. Arch Int Med 46: 137-160 (July) 1930

Altnow H C, Van Winkle Charlotte C, Maly H W, and Williams L E. Renal Amyloidosis. Clinical Course and Pathologic Lesions in Sixteen Cases. Arch Int Med. 56: 944-975 (Nov) 1935

²Smith F L, 2nd, Rathmell T K, and Marcol G E. The Early Diagnosis of Acute and Latent Plumbism. Am J Clin Path 8: 471-514 (Sept) 1938

identification of the compound in the urine and the history of the cause differentiate these conditions from acute glomerular nephritis

IX TYPES OF UREMIA

A True Uremia¹—This is characterized clinically by headache, stupor increasing gradually to coma, and frequently by a pericarditis and colitis. Convulsions may or may not occur. There is always a very high urea nitrogen (over 60 mg). It occurs in chronic diffuse nephritis as a rule, in subacute glomerular nephritis frequently, and in acute glomerular nephritis and hypertensive cardiovascular renal disease occasionally. It is a common termination of hydronephrosis and pyelonephritis.

B Acute Cerebral Edema—This is sometimes called eclamptic uremia because of the characteristic convulsions and coma in a patient with high blood pressure. Impaired renal function may or may not be associated. The cerebrospinal fluid pressure is increased. It occurs in eclampsia, in acute or subacute glomerular nephritis, and occasionally in chronic glomerular nephritis and in hypertensive cardiovascular disease. It is differentiated from true uremia by the fact that the blood urea nitrogen is usually under 60 mg per 100 cc, and by the prompt recovery with elevation of the head and relief of the intracranial pressure by hypertonic sucrose or sorbitol intravenously. The acute cerebral edema is due to the hypertension itself and not to the impaired kidney function.

C Cerebral Vascular Accidents—Because these are common in patients with hypertension and may result in coma or convulsions without the specific localizing signs, the term pseudo uremia has been used to indicate the clinical similarity of the picture to true uremia. The differentiation is made by the blood urea nitrogen, which is under 60 mg per 100 cc, and the cerebrospinal fluid and physical findings.

D Acidosis or Alkalosis—Acidosis or alkalosis often cause coma in patients with impaired renal function. An alkali reserve of under 30 or over 100 and a blood urea nitrogen under 60 mg per 100 cc will differentiate these from true uremia. Acidosis or alkalosis may be a complication of true uremia.

Because of failure to differentiate correctly the conditions listed under B, C, and D from true uremia, some clinicians have failed to

¹Mason M F, Resnik H, Minot A S, Rainey J, Pilcher C and Harrison T R. Mechanism of Experimental Uremia. Arch Int Med 60: 312-336 (Aug) 1937.

recognize the value of blood urea nitrogen estimations in the diagnosis of uremic coma

Σ SUMMARY OF THE INDICATIONS FOR THE MORE IMPORTANT LABORATORY TESTS IN DISEASES OF THE URINARY SYSTEM

A In all cases in which disease of this system is suspected a routine urinalysis and a routine hematologic examination should be performed

B In all cases in which uremic breath, deep breathing (Kussmaul), *hypertension*, diffuse edema, retinopathy, albuminuria with casts, obscure anemia, or history of alterations in the urine output suggest impaired renal function the blood urea nitrogen and the 24 hour urine volume should be determined. Ideally, the phenolsulphonphthalein excretion and the urea clearance should be determined also

C In all chronic cases of suspected impaired renal function (B above) without edema which have a blood urea nitrogen under 50 mg per 100 cc and show no evidence of impending cerebral edema or cardiac decompensation, the dilution and concentration tests should be done

D In all cases in which the blood urea nitrogen is over 20 mg per 100 cc the level of the alkali reserve and, ideally, of the blood "creatinine" should be determined

E In all cases in which pus in the catheterized urine indicates infection in the urinary tract, the causative organism should be identified by Gram and Ziehl Neelsen stains, culture, and guinea pig inoculation. Treatment should be controlled by pH and sulfanilamide determinations on the mixed 24 hour urine

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CHAPTER III

DISORDERS OF CARBOHYDRATE, PROTEIN AND FAT METABOLISM WITH ESPECIAL REFERENCE TO DIABETES MELLITUS AND DISTURBANCES OF ACID-BASE EQUILIBRIUM

I RESUMÉ OF THE ESSENTIAL POINTS IN THE NORMAL AND PATHOLOGIC PHYSIOLOGY AND BIOCHEMISTRY OF CARBOHYDRATE METABOLISM¹

Carbohydrate food is normally absorbed in the form of the monosaccharides which are carried with the portal blood to the liver where they are transformed to glycogen. If absorption is rapid a portion may pass through the liver unchanged, thus elevating the level of the blood sugar in the systemic circulation. In addition, glycogen is formed from the glycerol of fat² and from certain amino acids among which are alanin, arginin, aspartic acid, cystin, glutamic acid, hydroxyglutamic acid, glycin, methionin, isoleucin, norleucin, prolin, oxyprolin, and serin. Fat may, therefore, ultimately give rise to dextrose (d-glucose³) equal to about 10 per cent of the weight of the fat absorbed, and protein may give rise to dextrose equal to about 58 per cent of the amount of protein absorbed. As the relative proportion of glycerol to total fat and of glycogen-forming amino acids to total protein varies in different types of fat and protein it is evident that the figures given can be merely average figures subject to very considerable variations with changes in diet. If calculated from the protein or fat ingested still greater variations will occur, due to differences in absorption. A possible further source of error is a smaller formation of glycogen and thus of dextrose than the figures suggest, for they are based on experiments carried out under conditions designed to secure the greatest possible demand for dextrose and hence are surely maximum figures. There is little evidence to show that the transformation to dextrose occurs to this extent under conditions when the demand for dextrose is less extreme. The glycogen of the liver serves as a store-

¹Lusk G. The Science of Nutrition. Pp 319-399 and 650-657. W. B. Saunders Company Philadelphia. Ed 4. 1928.

Peters J. P. and Van Slyke D. D. Quantitative Clinical Chemistry. Vol I pp 70-217. Williams and Wilkins Co. Baltimore. 1931.

Best C. H. and Taylor N. B. The Physiological Basis of Medical Practice. Pp 864-958. William Wood and Co. Baltimore. 1937.

²The author agrees with Lusk (footnote 1) that notwithstanding its distinguished advocates the view that dextrose is derived in the body from the non glycerol portion of fat is untenable. A summary of the experiments designed to show that carbohydrate is derived from the non glycerol portion of fat will be found in The Fuel of Life J. J. R. MacLeod Princeton University Press 1938 pp 147.

³Glucose is defined in the tenth revision of the Pharmacopoeia of the United States as a product obtained by the incomplete hydrolysis of starch. It consists chiefly of dextrose (d glucose) $[C_6H_{12}O_6]$ maltose, dextrins and water. The term dextrose should be reserved for the pure chemical compound d glucose.

LABORATORY DIAGNOSIS

house from which dextrose is formed at a rate sufficient to keep the level in the circulating blood remarkably constant when the extreme variations in the supply and demand for this sugar are considered. This level of dextrose in the blood in the morning before breakfast is 60 to 100 mg per 100 cc or reducing substances equivalent to 80 to 120 mg of dextrose as tested by current methods. Dextrose is in turn withdrawn from the blood by the tissues to be stored as muscle glycogen or changed to fat and then stored, or to be oxidized through as yet disputed intermediary substances to carbon dioxide and water. This oxidation furnishes energy equivalent to 4.0 calories per gram. Insulin, the internal secretion of the islets of Langerhans of the pancreas, is essential both for the formation of liver glycogen and for the oxidation of dextrose. Its exact chemical composition and mode of action are still unsettled.

Release of dextrose into the systemic circulation is effected by rapid absorption from the intestine, and from glycogenolysis caused by epinephrin, by stimulation of the splanchnic nerves, or by increased hydrogen ion concentration of the blood plasma such as may occur in acidosis from any cause. Increased withdrawal of dextrose from the blood stream occurs when the demand for energy is increased in the presence of adequate insulin, and in the presence of excess insulin. The level of the blood sugar at any moment is therefore, the result of these two opposing tendencies. Normally it rises after each meal to a peak of not more than 150 mg per 100 cc at the height of absorption, to fall again to below 120 mg by the next meal and to the lowest level reached just before breakfast in the morning.

If the level of the blood sugar is raised above a certain point in any individual, dextrose appears in the urine. This level at which dextrose first begins to spill over into the urine is called the renal threshold. It is normally between 125 and 220 mg per 100 cc (105 and 200 mg true dextrose). Pathologically it may be either higher or lower than this. Obviously, the level of the renal threshold can be ascertained only by a determination of the blood sugar level at the paroxysmal moment when dextrose first appears in the urine. It is also obvious that dextrose may be excreted in the urine only for a relatively short time at the height of absorption and, therefore, a mild glycosuria will be most apt to be detected if a mixed 24 hour sample is tested and most apt to be missed if only a morning specimen is examined.

In diabetes mellitus the fundamental difficulty is a deficient supply of insulin, which in turn results in deficient glycogen storage and deficient oxidation of dextrose. The unutilized dextrose accumulates in the blood stream until the renal threshold is exceeded and then appears in the urine.

Other disturbances of carbohydrate metabolism are chiefly of endocrine origin and their mechanism is not clear. It has been shown by Houssay and others¹ that the anterior lobe of the pituitary produces hormones which modify carbohydrate metabolism. If the anterior pituitary is removed in diabetic animals, they survive longer and show less marked evidence of insulin deficiency, if extracts of the anterior lobe of the pituitary are given to such animals, the diabetic syndrome becomes more severe. If epinephrin is given to an animal or

¹ Houssay B A Hypophysis and Metabolism New England J Med 214 961-971 (May 14) 1936
Long C N H The Influence of the Pituitary and Adrenal Glands Upon Pancreatic Diabetes The Harvey Lectures Williams and Wilkins Co Baltimore 1936-1937

person with a good store of glycogen in the liver, glycogenolysis is hastened and hyperglycemia occurs

II THE URINE VOLUME AND SPECIFIC GRAVITY

These have been fully discussed (Chapter II) It is, therefore, only necessary to reiterate that a large volume of pale urine with a disproportionately high specific gravity is suggestive of diabetes mellitus, but the volume and specific gravity are often normal In diabetes insipidus a very large volume (4 to 30 liters) of low specific gravity is excreted which does not give a positive test with Benedict's qualitative reagent It is a disorder of water and salt excretion in which the kidney apparently loses its power to concentrate the urine and is due to disease or injury of the posterior lobe of the pituitary body or of the brain in that region

III REDUCING SUBSTANCES IN URINE

Glycosuria is the excretion of dextrose in the urine Reducing substances equivalent to about 0.03 to 0.10 per cent of dextrose are normally excreted Most of this is made up of non fermentable substances There is some evidence to suggest that about 0.01 per cent of dextrose is normally excreted ¹

A *Benedict's Qualitative Test*—This should be done as a routine and preferably on a portion of the mixed 24 hour sample if it is desired to detect the milder grades of glycosuria The reagent is reduced by dextrose, levulose, lactose, galactose, maltose, and pentose, and also by the non carbohydrate substances glycuronic acid, formaldehyde (after taking urotropin), homogentisic acid (alkaptonuria) and dihydroxyphenylalanine (tyrosinosis) Homogentisic acid quickly darkens on exposure to oxygen so that the urine turns black shortly after it is voided It appears only in the rare congenital disorder of tyrosin metabolism known as *alkaptonuria* This disease is not incompatible with a life of normal length and comfort

Tyrosinosis² is a similar disorder of tyrosin metabolism, but in this condition the reducing substance does not darken on exposure to air

¹ West E S and Peterson V L The Sugars of Urine I Determination of the Reducing Sugars of Urine Biochem J 26 1720-1727 1932

West E S Lange A C and Peterson V L The Sugars of Urine II Factors Affecting the Excretion of Fermentable and Non fermentable Sugars in Urine Biochem J 26 1728-1741 1932

West E S and Steiner A The Sugars of Urine III The Chemical Nature of the Fermentable Sugar of Normal and Starvation Urine Biochem J 26 1742-1749 1932

² Medes Grace A New Error of Tyrosine Metabolism Tyrosinosis the Intermediary Metabolism of Tyrosine and Phenylalanine Biochem J 26 917 1932

Chloral, chloroform, morphine, camphor, the balsams, aminopyrine and salicylic acid and its derivatives are the more commonly used drugs which are excreted in combination with glycuronic acid. Glycuronates do not give osazones and are not fermented by yeast. Since they cease to be excreted after stopping the drug, differentiation from dextrose is easy if considered.

The fallacy of considering a positive reduction test proof of the presence of sugar, as is commonly done, is, therefore, obvious. Charts and report forms should contain a heading "reduction" for the reporting of the results of this test, and the heading "sugar per cent" should be reserved for the name of the substance found to be causing the reduction and the results of quantitative estimation.

In diabetes mellitus under treatment when only a small amount of dextrose is found in the 24 hour sample it is frequently desirable to test fractional samples of urine collected for short periods of 1 to 2 hours throughout the day for reduction, as the total dextrose excretion may be occurring during one short period and a slight change in the time of a meal hour or dose of insulin or readjustment of the relative proportions of the insulin dose and the meal preceding this excretion will enable the clinician to keep the urine sugar free without altering the total diet or the total insulin dosage.

B Identification of the Reducing Substance—This should be done the first time a urine from a given patient shows reduction. It is usually unnecessary later as there is little likelihood that a person will excrete dextrose at one examination and a different sugar later. Lactose, of course, need only be tested for in the urine of pregnant or lactating women, infants or others on a milk diet.

C Interpretation—1 **Dextrose**—(a) *Diabetes mellitus* is the most common and important cause of glycosuria, but in early diabetes and in cases complicated by nephritis, glycosuria may be absent. It is also absent in some cases of severe diabetes with a high renal threshold for dextrose. The percentage of dextrose may vary from a trace to 10 per cent.

Under the term diabetes mellitus, as hereafter used, are grouped all conditions in which a deficiency of insulin occurs. They may all show the laboratory findings characteristic of diabetes mellitus with, in some cases, additional findings also. The members of this group are

(1) Juvenile diabetes mellitus (a congenital deficiency of islet tissue?)

(2) The milder diabetes mellitus of older persons. This is based on various types of fibrosis, atrophy, arteriosclerosis, arteriolosclerosis, or other as yet ill defined pathologic changes in the pancreas.

(3) Acute, subacute, or chronic pancreatitis This may give rise to a transient or permanent picture of diabetes mellitus This group undoubtedly accounts for the cases attributed to chronic cholecystitis

(4) Benign or malignant tumors of the pancreas These not infrequently produce the syndrome of diabetes mellitus If a pancreatic tumor has been diagnosed, the presence of this syndrome usually indicates that the tumor growth involves the majority of the pancreas and is, therefore, very extensive Absence of this syndrome should not be considered as evidence against the diagnosis of tumor

(5) Hemochromatosis or bronzed diabetes is a rare disease characterized by enlargement of the liver and spleen, brownish pigmentation of the skin with the blood and urine findings characteristic of diabetes mellitus At least some of these cases fail to respond to insulin therapy

(b) *Temporary Glycosurias* —These are usually non diabetic The causes are

(1) Alimentary glycosuria This occurs after excessive carbohydrate ingestion It is exceedingly difficult to produce in the normal individual, a dosage of 150 to 500 gm of pure dextrose being required Hence, an alimentary glycosuria is always an indication for a dextrose tolerance test, as alimentary glycosuria may prove to be the earliest clue to an incipient diabetes mellitus, or other disorder of carbohydrate metabolism

(2) Severe chilling of the body

(3) Pregnancy In the first three months it is usually not significant Later in pregnancy a positive reduction test is most apt to be due to lactose in the urine If dextrose is demonstrated, true diabetes mellitus may be the cause Therefore, a fasting blood sugar estimation and if that is normal a dextrose tolerance test should always be performed if glycosuria is found later than the third month of pregnancy It is safer thus to determine the cause of glycosuria in the first three months although it will usually be found to be due to a low renal threshold which is not pathologically significant

(4) After intravenous saline injection ✓

(5) Asphyxia ✓

(6) Drugs Adrenalin produces glycosuria by liberating dextrose from glycogen reserves Anxiety, anger, or fright may produce a temporary glycosuria due to excessive liberation of epinephrin from the adrenals (Cannon) Thus after a severe examination an appreciable percentage of a class may show dextrose in the urine This is of no clinical significance

Anesthetics, morphine and carbon monoxide not infrequently give rise to glycosuria by producing asphyxia

Phloridzin reduces the renal threshold to such an extent that the glycogen reserves can be almost completely exhausted The dextrose is removed from the

body so rapidly that almost no oxidation occurs. It is, therefore, a valuable aid to the physiologist in studying carbohydrate metabolism but practically never needs to be considered in the clinical differential diagnosis of glycosuria. Its mode of action is apparently a complete inhibition of the ability of the renal tubule to reabsorb dextrose from the urine.

(7) Injury to the central nervous system, either traumatic or due to cerebral vascular accidents (cf. sugar puncture of Claude Bernard). This is important because these patients are often first seen when in coma. The finding of dextrose in the urine (often associated with ketone bodies) may lead one in some cases to make an erroneous diagnosis of diabetic coma if this possibility is not considered. (See chapter X.)

(c) Persistent glycosurias which are not true diabetes mellitus

(1) Hyperthyroidism. This possibility should be considered in the case of a patient who shows a persistent or alimentary glycosuria not otherwise explained. A dextrose tolerance test and a basal metabolic rate determination should be done. Since glycosuria is absent in many cases, and a true diabetes mellitus may co-exist with hyperthyroidism, further study is necessary, and often a sub-total thyroidectomy must be performed before a final decision can be made as to whether hyperthyroidism alone was responsible for the finding.

(2) Hyperpituitarism. This may produce similar results but such a glycosuria is still less common than that of hyperthyroidism.

(3) Severe glomerular nephritis or chronic diffuse nephritis with marked nitrogen retention and acidosis. In such cases slight glycosuria due to a combination of high blood sugar and lowered renal threshold may occur. This does not alter the prognosis and probably results from excessive glycogenolysis secondary to the acidosis. It does not occur in nephrosis or in mild glomerulo-nephritis. In cases of hypertensive cardiovascular renal disease, glycosuria may occur, due in most cases to the co-existence of a mild diabetes mellitus on the basis of vascular changes in the pancreas identical with those occurring in the kidney and other internal organs.

(4) Renal glycosuria, renal diabetes, normoglycemic glycosuria or diabetes innocens. In this condition the glycosuria may be constant and is due to a low renal threshold for dextrose. The patient's health is not affected since the ability to oxidize dextrose is not impaired.

2 Levulose¹ (fructose).—This may occur with dextrose in severe diabetes mellitus or alone in a very rare disorder of metabolism in which a definite percentage of all ingested levulose is excreted in the urine unchanged. The prognosis is good.

¹ Heeres P. A. and Vos H. Fructosuria. Arch. Int. Med. 44: 47-64 (July) 1929.

An alimentary levulosuria occurs in some diffuse diseases of the liver such as cirrhosis or subacute hepatitis (catarrhal jaundice). The ingestion of levulose was even suggested as a test of liver function but proved unreliable.

3 **Lactose**—This occurs in the later months of pregnancy and during lactation and occasionally in infants after too rapid absorption of lactose. Its only importance is that unless tested for it might be confused with dextrose. Dextrose and lactose may occur together. If dextrosazone crystals are secured together with a positive lactose test the quantity of dextrose present may be determined by quantitative sugar estimations before and after fermentation with yeast or preferably with *Salmonella shottmulleri* (*Bacillus paratyphosus* B). The difference between the two estimations is due to dextrose.

4 **Galactose**—This may occur in the urine of nursing infants or, in rare instances, in severe liver disease after the ingestion of much lactose (milk). The excretion of over 3 gm. of galactose in the urine (See Chapter V) after oral administration of 40 gm. is considered by R. Bauer to be diagnostic of impaired liver function.

5 **Maltose**—This is very rarely excreted in the urine. It is said to occur in some cases of interstitial pancreatitis.

6 **Pentose**—Pentosuria is rare.

(a) *Alimentary pentosuria* occasionally occurs after excessive ingestion of food containing pentose (e.g. cherries).

(b) *In diabetes*, along with dextrose, pentoses may rarely be found.

(c) *Idiopathic pentosuria*¹ is a congenital anomaly of metabolism of no clinical significance provided it is recognized and the patient is not treated for diabetes mellitus. L-xyloketose is the pentose usually present.

7 **Pentosans**—These rarely occur. They are the basis of the so called Cambridge reaction which was supposed to indicate disease of the pancreas. The test is no longer used.

D Quantitative Estimation of Dextrose—This should be done daily or at frequent intervals on an accurately collected 24-hour sample of urine on every patient with diabetes mellitus who shows more than a one plus reduction of Benedict's solution. It is of little or no value if done on inaccurately collected, improperly preserved, or single specimens. It is occasionally of value in research studies on other types of glycosuria. The results should be reported both as percentage and as total number of grams excreted per day.

IV THE FASTING BLOOD SUGAR

This should be determined on each patient when dextrose is first found in his urine and on any patient in whom diabetes is suspected even though sugar is not present in the urine. It should be repeated

¹ Margolis J. I. Chronic Pentosuria and Migraine. *J. A. M. A.* 93: 173-175 (July 20) 1929.

Franklewitz M. Studies in Pentosuria. *Am. J. Med. Sci.* 186: 539 (Oct.) 1933.

Sunderman F. W. Essential Pentosuria. *M. Clin. North America* 21: 1245-1225 (July) 1937.

daily until the blood sugar has returned to normal and thereafter, once a week to once a month throughout the patient's life to determine the accuracy of clinical control. In a diabetic, an acute infection or exacerbation of symptoms is an indication for a blood sugar estimation. In patients receiving insulin¹ the blood sugar level as determined on a specimen of blood taken before breakfast is apt to be the highest which has occurred at any time during the preceding 24 hours, whereas in all other cases it is usually the lowest level reached.

A Normals—The fasting value by the commonly used methods is 80 to 120 mg of "dextrose" per 100 cc of blood. It is very constant in health. Work² with methods more specific for dextrose than those in common use has shown that the actual fasting dextrose level of blood is almost exactly 21 mg lower and that the higher figures by the usual methods are due to other reducing substances. This does not alter the diagnostic value of the blood sugar estimation by the tests now in use. Hence, all interpretations given here are for methods which give values of 80 to 120 mg per 100 cc on the blood of normal persons. Blood sugar values are sometimes reported in percentage figures, e g, 0.12 per cent is the same as 120 mg per 100 cc. True dextrose values may be obtained from the values given in this book by subtracting 21 mg.

B Hyperglycemia—This is a fasting blood sugar content above 120 mg. An estimation above 150 mg is definitely pathologic. The causes are

1 **Diabetes Mellitus**—This is by far the most common and the most important cause. A diagnosis of diabetes mellitus is justified even with no glycosuria if no other cause for the hyperglycemia is found.

2 **Severe Nephritis**

3 **Hyperthyroidism** (some cases)

4 **Hyperpituitarism** (in a small proportion of cases)

5 **Cholecystitis** (probably due to an associated pancreatitis)

6 **Pancreatitis**

7 **Infections**

Note. A true diabetes mellitus may occur in association with any of these conditions.

¹ This applies either to regular insulin, to protamine zinc insulin or to the other slow acting insulins as usually administered.

Joslin E P. Protamine Insulin. J A M A 109 497-503 (Aug 14) 1937.

² Somogyi, M. A Method for the Preparation of Blood Filtrates for the Determination of Sugar. J Biol Chem 86 655-663 (April) 1930.

Somogyi, M. Distribution of Blood Sugar between Corpuscles and Plasma in Diabetic and in Alimentary Hyperglycemia. Arch Int Med 42 931-938 (Dec) 1928.

Campbell R A, Osgood E E, and Haskins H D. Normal Renal Threshold for Dextrose. Arch Int Med 50 952-957 (Dec) 1932.

8 Hemochromatosis

9 Essential hypertension associated with obesity Probably in such cases there is a true diabetes mellitus of mild grade due to changes in the pancreas similar to those which usually occur in the kidney

10 Transient Hyperglycemia—This may occur in any of the cases of temporary glycosuria listed above, with the exception of renal diabetes, the effects of phloridzin and most cases of glycosuria in the first three months of pregnancy

C Hypoglycemia¹—This is a blood sugar content below 80 mg per 100 cc It should be suspected and the blood sugar should be estimated in any patient showing the syndrome of sweating, hunger, tremor, and later convulsions and coma which is usually called a hypoglycemic reaction,² or in any patient presenting evidence suggestive of the conditions listed below If the blood sugar gets as low as 45 mg convulsions usually occur The causes are

1 Overdosage of insulin, or failure to ingest or absorb the amount of carbohydrate calculated for the insulin dosage This is the most common cause of hypoglycemia With the slow acting insulins hypoglycemia may occur during the night or after exercise The usual symptoms are headache and nausea but coma may occur without the patient awaking

✓2 Renal Glycosuria—Persistent glycosuria due to a low renal threshold for dextrose is diagnostic of this condition

3 Endocrine Hypofunction—(a) *Myxedema or Cretinism* (b) *Addison's Disease*³ (c) *Pituitary Disease*

4 Hyperinsulinism⁴—This is being recognized with increasing frequency and should be suspected not only when a typical hypoglycemic reaction occurs, but also when periodic neurologic attacks occur at the time of day that the blood sugar is lowest The commoner forms are epileptiform seizures, headaches, psychotic attacks, pareses, narcolepsy, and periods of amnesia, confusion or stupor The patients often discover that they can relieve or prevent the attacks by taking

¹ Cragg R W Power M H and Lindem M C Carcinoma of the Islands of Langerhans with Hypoglycemia and Hyperinsulinism Arch Int Med 60 88-99 (July) 1937
Hartmann A F Jaudon J C and Morton Marie Hypoglycemia J Pediatrics 11 1-36 (July) 1937

² It is now known that a hypoglycemic reaction depends not so much on how low the blood sugar falls as on how rapidly it falls For example the blood sugar can be gradually reduced to much below normal without a hypoglycemic reaction and on the other hand in a patient with a hyperglycemia a sudden fall in blood sugar may produce a typical reaction even though the final level reached is still above normal

³ Welty J W and Robertson H F Hypoglycemia in Addison's Disease Am J Med Sc 192 760-764 (Dec) 1936

⁴ Harris S Epilepsy and Narcolepsy Associated with Hyperinsulinism Report of Three Cases of Epilepsy and One Case of Narcolepsy Cured Clinically by Partial Resection of Body and Tail of Pancreas J A M A 100 321-327 (Feb 4) 1933

B Pathologic Results—These occur in the following conditions

1 **Diabetes Mellitus**—This is by far the most common and important cause of an abnormal curve. The curve rises sooner and higher and stays up longer than in normals. The high point is often at one hour and usually it goes above 200 mg and has not returned to normal at the end of 2 hours. Dextrose does not appear in the urine until the blood sugar reaches 125 to 220 mg and may not appear even then, since in many cases of diabetes the renal threshold is raised.

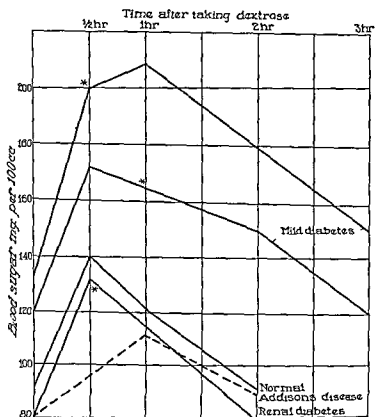


FIG. 1.—Blood sugar curves secured in dextrose tolerance tests. *Point at which glycosuria begins

2 **Endocrine Disturbances**—(a) *Hyperfunction*—The curve is high and resembles that in diabetes (although it is usually not so markedly abnormal) in

Hyperthyroidism¹

Acromegaly and gigantism in the active stage, or hyperfunction of the pituitary. A hypofunction of the pituitary is apt to occur later with a corresponding alteration in the curve.

¹ John H. J. Hyperthyroidism Showing Carbohydrate Metabolism Disturbances. Ten Years Study and Follow Up of Cases. J. A. M. A. 99:620-627 (Aug. 20) 1932.

In these conditions, although the curve is high, the sugar tolerance is said to be low because glycosuria may occur after relatively small doses of dextrose

(b) *Hypofunction*—The curve is low and resembles that in renal diabetes (but is *not* associated with glycosuria) in

Myxedema and cretinism

Addison's disease

Hypofunction of the pituitary

In the above conditions, although the curve is low, the sugar tolerance is said to be high because enormous amounts of dextrose may be ingested without glycosuria

3 **Renal Glycosuria**¹—In this condition the curve is low, seldom going above 140 mg and dextrose appears in the urine when the blood sugar level is less than 125 mg in other words, the renal threshold is low

To diagnose renal diabetes it must be shown that dextrose is appearing in the urine at a time during which the blood sugar level does not exceed 125 mg Therefore the blood sugar estimation must be done on blood collected at the same time (within 15 minutes, not merely on the same day) as the urine sample which contains dextrose The dextrose tolerance test offers the best opportunity to do this Early pregnancy must of course, be excluded Variations in carbohydrate intake should produce relatively slight variations in the quantity of dextrose excreted in the urine, as demonstrated by the dextrose oxidizing ability test

4 **The First 12 Weeks of Pregnancy**—During this period, the changes described for renal glycosuria may occur

5 **Severe nephritis**² of the types showing nitrogen retention These cases may give a diabetic type of curve with or without glycosuria It is important to consider this before making the diagnosis of diabetes in a nephritic The two conditions may co exist

6 **Infections**³—During many types of infections (particularly infectious arthritis) a slightly abnormal curve approaching the diabetic type occurs This is of no diagnostic significance but suggests that insulin may be of therapeutic value in such cases

¹ Marble A Renal Glycosuria Am J Med Sci 183 811-826 (June) 1932

² Smith Florence H and Smith K A Normoglycemic Glycosuria Differentiated from Other Benign Glycosuria and Diabetes Mellitus Arch Int Med 60 119-132 (July) 1937

³ Linder C C Hiller A and Van Slyke D D Carbohydrate Metabolism in Nephritis J Clin Invest 1 24-32 (Feb) 1925

⁴ Williams J I and Dick C F Decreased Dextrose Tolerance in Acute Infectious Diseases Arch Int Med 50 801-818 (Sept) 1914

⁵ Schmidt I C Eastland J S and Burns J H Infection and the Tolerance for Dextrose Arch Int Med 54 466-481 (Sept) 1934

7 **Carcinoma** (especially of the digestive tract) —This often gives a high curve and some believe it is sufficiently constant to be of diagnostic value

8 **Following a Low Carbohydrate Diet**¹—If such diets are ingested for a few days, even normal persons will give a diabetic type of curve. It is important, therefore, that persons who are to have a dextrose tolerance test be on a liberal carbohydrate regimen for several days

9 **Hyperinsulinism**—Dextrose tolerance curves may be of any type, although a low value at some stage in the test is the rule. The high curve occasionally obtained suggests that there is a faulty regulation of the output of insulin in some cases rather than a consistently high secretion. This syndrome is sometimes called dysinsulinism. The diagnosis of hyperinsulinism should be based on the finding of a blood sugar of 50 mg per 100 cc or less at the time of the attack, not on the results of a dextrose tolerance test

10 **Faulty Absorption**—Bizarre curves in which the level remains constant or decreases and then goes up may result from delayed absorption due to nausea, emotional upsets, gastrointestinal disease, etc

11 **Von Gierke's Glycogen Disease**—The curve is low and glycosuria does not occur

VI THE ONE HOUR, TWO DOSE DEXTROSE TOLERANCE TEST²

This test was developed by Exton and Rose³ and is simpler than the preceding dextrose tolerance test. It is satisfactory for diagnosis of diabetes mellitus but is not so satisfactory in other endocrine disturbances

A Normals—The first blood sugar is less than 120 mg per 100 cc, the half hour level is less than 50 mg above the first value, and the one hour level is less than 30 mg above the half hour value

B Pathologic Results—Diabetes mellitus is probably present if any two of the following deviations from normal in the test are found

- (1) A first blood sugar of over 120 mg per 100 cc
- (2) A half hour value of more than 50 mg per 100 cc above the first level
- (3) A one hour level of 30 mg per 100 cc above the half hour level

¹ McCullagh F. P. and Johnston C. R. K. Manipulation of Glucose Tolerance by Diet. *Am J M Sc* 195 773-781 (June) 1938

² Cooperstock M. and Galloway Josephine M. One Hour Two Dose Dextrose Tolerance Test. *Am J Dis Children* 55 1221-1232 (June) 1938

³ Exton W. G. and Rose A. R. The One Hour Two Dose Dextrose Tolerance Test. *Am J Clin Path* 4 381-399 (Sept) 1934

The normal values and interpretation have been given according to the criteria of Gould, Altschuler and Mellen¹ since these have been found more satisfactory than the original criteria of Exton and Rose. The interpretation is the same in children if the test is performed as recommended.

VII QUANTITATIVE METHODS FOR DETERMINING THE SEVERITY OF DIABETES MELLITUS

These tests are of value in following the course of treated diabetes.

A Dextrose Oxidizing Ability Test—This test should be done at intervals on all diabetic patients who are under control but do not require more than 20 units of insulin a day. It is also desirable to perform it on patients with renal glycosuria.

1. Technic—No insulin is given during the period of the test. The patient is placed on a weighed diet, the total dextrose equivalent of which is calculated from this formula: 100 per cent of the carbohydrate plus 58 per cent of the protein plus 10 per cent of the fat² in the diet (absorbed) equals the total dextrose value. The protein absorbed may be more accurately calculated by multiplying the total nitrogen of the 24 hour urine by 6.25. Quantitative dextrose estimations on the 24 hour urine are made daily. After they become relatively constant the average daily excretion is subtracted from the average daily intake. The difference is the grams of dextrose the patient is able to metabolize with his own insulin. This figure is often called the patient's *carbohydrate tolerance*. This must not be confused with the dextrose tolerance test.

In renal glycosuria increasing the dextrose value of the diet increases only slightly the dextrose excreted in the urine. In diabetes mellitus, increasing the dextrose value of the diet increases the dextrose excreted in the urine by that amount. This is the most important point in the differential diagnosis between renal glycosuria and diabetes mellitus.

If there is much daily variation after the first 48 hours, it is probable that the patient is obtaining extra food or that the collection of the 24 hour urine specimens is inaccurate.

B Insulin Coefficient³—This method is applicable to patients with severe diabetes to whom regular insulin is being administered.

¹ Gould S. E., Altschuler S. S. and Mellen H. S. The One Hour Two Dose Glucose Tolerance Test in the Diagnosis of Diabetes Mellitus. *Am J M Sc* 193 611-617 (May) 1937.

² See footnote 2 p. 55.

³ Williams J. R. The Insulin Coefficient: an Improved Method for the Clinical Control of Diabetes Mellitus. *Ann Int Med* 5 264-273 (Sept) 1931.

1 **Technic**—Reduce the insulin dosage until 5 to 20 gm of dextrose are excreted in the urine daily. When the dextrose excretion has become constant, determine the diet dextrose and the urine dextrose as outlined above. The difference is the dextrose utilization (DU). Since, under the conditions of this test, one unit of insulin will metabolize about 4 gm of dextrose, $DU/4 =$ units of insulin required (IR). The insulin required minus the insulin administered (IA) equals the insulin coefficient (IC) or the actual amount of insulin which the patient is producing. It is obvious that the diet given must be adequate to cover the patient's caloric requirements.

C Interpretation

TABLE 5—INTERPRETATION OF THE INSULIN COEFFICIENT AND DEXTROSE OXIDIZING ABILITY TESTS

	Insulin coefficient	Dextrose oxidizing ability
Normals	100 or more	400 gm or more
Borderline diabetes	75 to 100	300 to 400 gm
Mild diabetes	40 to 75	160 to 300 gm
Moderate diabetes	25 to 40	100 to 160 gm
Severe diabetes	Less than 25	Less than 100 gm
Total diabetes	0	0

It is obvious that the dextrose which can be oxidized expressed in gm is four times the Insulin Coefficient.

VIII KETOSIS¹

This is the formation of the ketone bodies, i.e., acetone, acetoacetic acid, or beta-hydroxybutyric acid, in such quantities that they are excreted in the urine. Acetone is present alone in mild cases and in severe cases in association with acetoacetic acid or with both of the other ketone bodies. It must not be confused with acidosis.

A Chemical Formulae

Acetone CH_3COCH_3

Acetoacetic acid $\text{CH}_3\text{C}(\text{O})\text{CH}_2\text{COOH}$

Beta-hydroxybutyric acid $\text{CH}_3\text{CHOHCH}_2\text{COOH}$

B **Mode of Production**—Acetoacetic acid is formed first. Acetone is derived from it by loss of CO_2 , and beta-hydroxybutyric acid is produced from it.

¹Lusk, G. The Science of Nutrition. Pp 662-682. W. B. Saunders Philadelphia Ed 1 1928.

Woodyatt, R. T. Objects and Method of Diet Adjustment in Diabetes. Arch Int Med 28 125-141 (Aug) 1921.

Shaffer, P. A. Antiketogenesis. J Biol Chem 54 399-441 (Oct) 1922.

by a process of reduction. Acetoacetic acid is a normal intermediate product in the oxidation of the acids that occur in combination with glycerol as fats, and of certain amino acids (leucin, tyrosin, phenylalanin, and histidin) from proteins. In normal metabolism it is quickly oxidized to carbon dioxide and water and so is never excreted in the urine. However, it has been shown that dextrose must be oxidized in order that this complete oxidation of a ketone acid may occur, and also that there is a quantitative relation between the amount of dextrose which is being oxidized and the quantity of acetoacetic acid that can be oxidized. For every molecule of dextrose oxidized one molecule of acetoacetic acid can be completely oxidized. Hence, if the metabolism can be so adjusted that one or more molecules of dextrose is oxidized for each molecule of acetoacetic acid that is formed, ketosis will not occur. If more than one and less than two molecules of acetoacetic acid are formed for each molecule of dextrose that is being oxidized, slight ketosis occurs although some of the excess acetoacetic acid beyond one molecule is oxidized. If over two molecules of acetoacetic acid are formed for each molecule of dextrose oxidized, none of the excess acetoacetic acid beyond the two molecules is oxidized, and a marked ketosis results. Therefore, a diet which would result in such a ratio should never be prescribed unless a severe ketosis is desired (e.g., in the treatment of epilepsy or pyelitis).

As explained above, 100 per cent of the carbohydrate plus 58 per cent of the protein plus 10 per cent of the fat, equals the total dextrose value of the diet or the total antiketogenic substance in grams. About 90 per cent of fat is fat acid capable of producing acetoacetic acid, and 46 per cent of the weight of protein is equivalent to fat acid in its acetoacetic acid producing possibilities, that is to say, if 100 gm. of protein could be metabolized in the absence of dextrose (impossible because 58 gms. of dextrose would be formed from this amount of protein) the amount of acetoacetic acid produced would be the same as that produced from 46 gm. of fat acid or 51.1 gm. of fat. This does not imply that fat acid is ever produced from protein. The total ketogenic (acetoacetic acid producing) value of the diet is, therefore, 90 per cent of the fat plus 46 per cent of the protein. Ketosis occurs if more than one molecule of acetoacetic acid is formed for each molecule of dextrose oxidized. One molecule of fat acid will produce one molecule of acetoacetic acid. Therefore, to prevent ketosis the diet must be so adjusted that there is at least one molecule of antiketogenic substance for each molecule of ketogenic substance and enough insulin must be given so that all the antiketogenic substance is oxidized. The method of calculating ketogenic (K) and antiketogenic (AK) substances given above, however, gives results in grams not in molecules or gram molecules, but since the average molecular weight of fat acid is approximately 15 times the molecular weight of dextrose, an antiketogenic ketogenic ratio of 10:15 in grams is roughly equivalent to a ratio of 1:1 in gram molecules. The ketogenic and antiketogenic substances as calculated by the above methods are necessarily only rough approximations, since different fats and proteins will, of course, yield different quantities of dextrose and acetoacetic acid. Therefore, if ketosis is to be absolutely prevented, slightly less than 15 gm. (i.e., 14 gm.) of ketogenic substance should be given for each gram of antiketogenic substance in the diet. Since increasing the fat greatly increases the caloric value of the diet without much increase in the total dextrose value, a patient will require less insulin for a given caloric intake on a

A Buffer Action—A buffer solution is one which has the property of reacting with relatively large quantities of acid or alkali with only a very slight change in pH. There is, however, some change in pH even though it may not be measurable. A typical example of a buffer solution is a solution containing both acid and alkaline phosphates. If hydrochloric acid is added, some disodium phosphate is changed to monosodium phosphate and the ratio of hydrogen to hydroxyl ions does change slightly. The buffer action in this case depends on the replacement of the highly ionized acid (HCl) by the slightly ionized acid salt (NaH_2PO_4). The total replaceable hydrogen and, hence, the titration value of the resulting solution, is the sum of that in the buffer solution and that in the hydrochloric acid solution, but very much less of that hydrogen is in ionic form than in hydrochloric acid solution of the same titration value. The buffer action against alkalis is similar.

1 Buffer Substance in Plasma

(a) Buffers against Acid

- (1) Bicarbonate (does not buffer carbonic acid directly)
- (2) Alkaline phosphate (chiefly Na_2HPO_4)
- (3) Sodium proteinates
- (4) Ammonia (normally only trace present)

(b) Buffers against Alkali

- (1) Carbonic acid (carbon dioxide in solution)
- (2) Acid phosphate (chiefly NaH_2PO_4)
- (3) Proteins (not acid but will combine with alkali)

In emergencies ammonia is derived from proteins by deamination of amino acids and combines with acid substances. The ammonium salts are excreted by the kidney. Most of this ammonia formation occurs in the kidney itself.

The buffers against acid constitute the alkali reserve of the blood. The level of these buffers is indicated by the alkali reserve figure.

2 Buffers in the Red Cells—The importance of these has only recently been recognized. The most important of these is hemoglobin, itself, which, in the lungs, combines with oxygen to form a stronger acid than reduced hemoglobin and, in the tissues, reacts with carbon dioxide to form hemoglobin carbamate.¹ The reaction between hemoglobin and carbon dioxide is made possible by the catalyst, carbonic anhydrase, present in the red cells which accelerates in both directions the reaction $\text{CO}_2 + \text{H}_2\text{O} \rightleftharpoons \text{H}_2\text{CO}_3$. The potassium hemoglobin compound KHbO_2 is also important. This potassium hemoglobin compound is responsible for the buffering of 90 per cent of the carbon dioxide taken up by the blood. In addition the acid and alkaline potassium phosphates have some buffer value.

3 Acids to Be Neutralized

(a) *Volatile* (excreted by the lungs)—Carbonic acid, or carbon dioxide dissolved in plasma.

(b) *Fixed Acids* (excreted by the kidneys)—Lactic, acetoacetic, beta-hydroxybutyric, uric, hippuric, oxalic, sulphuric, phosphoric, mandelic and many other.

¹ Stadie W. C. and O'Brien Helen. The Carbamate Equilibrium. The Equilibrium of Oxyhemoglobin and Reduced Hemoglobin. *J Biol Chem* 117: 439-470 (Feb.) 1937.

Ferguson J. K. W. Carbamino Compounds of CO_2 with Human Hemoglobin and Their Role in the Transport of CO_2 . *J Physiol* 88: 40-55 (Oct.) 1936.

acids that may be accidentally or purposely ingested or injected Ammonium chloride, now used in treatment of edema in very large doses, acts as an acid. The foods that contain a preponderance of acid-producing substances are (a) meats, (b) eggs, (c) cereals, (d) cranberries, prunes, and plums, in other words the foods which contain much protein or phosphorus-containing compounds (oxidizing to H_2SO_4 and H_3PO_4), or fruits that yield unoxidizable organic acid (benzoic) in excess over the basic mineral substances present.

4 Alkalies to Be Neutralized —In normal metabolism there is never an excess of alkali produced and only rarely are alkaline substances ingested in disproportionate amounts, except as medicines. Therefore, the problem of neutralization of alkalies is much less important than neutralization of acids. The most commonly used drugs which are absorbed as alkalies are bicarbonates, acetates and citrates. Calcium carbonate and magnesium oxide may conserve the alkaline substances in the blood by combining with hydrochloric acid in the stomach, preventing the necessity of its neutralization in the duodenum. The foods that tend to increase the alkalinity are (a) fruits, with the exception of plums, prunes, and cranberries, (b) vegetables, especially soy beans and lima beans. These are foods which contain an excess of base over the acid-producing substances.

5 Mechanism of the Buffer Action —Since the red cell membrane is impermeable to sodium and potassium ions and to protein, the sodium and plasma proteins remain in the plasma while potassium and hemoglobin remain in the red cells, and only hydrogen, chloride and HCO_3 ions, and water and oxygen molecules pass through this membrane. The changes occurring in the tissues can be best illustrated by the following diagram.

Plasma	Cell wall	Cell contents
$H_2CO_3 \rightarrow (HCO_3)(H) \rightarrow$	H ions \rightarrow	$(H) + (Cl) + KHbO_2 \rightarrow KCl + HHbO_2 \rightarrow HHb + O_2$
$NaCl \rightarrow (Na) \downarrow (Cl) \rightarrow$ $\searrow NaHCO_3$	Cl ions \rightarrow	$(H) + (Cl) + K_2HPO_4 \rightarrow KCl + KH_2PO_4$
$H_2CO_3 \rightarrow (HCO_3)(H) \rightarrow$	H ions \rightarrow HCO_3 ions	$H_2CO_3 + HbO_2 \rightarrow HbNHCOOH + O_2 + H_2O$ $(H) + (HCO_3) + KHbO_2 \rightarrow KHCO_3 + HHbO_2 \rightarrow HHb + O_2$
$H_2CO + Na \text{ protein} \rightarrow$	H protein + $NaHCO_3$	$(H) + (HCO_3) + K_2HPO_4 \rightarrow KHCO_3 + KH_2PO_4$

In the lungs these reactions are exactly reversed because of the taking up of oxygen and the giving off of carbon dioxide, for example

Cell contents	Wall	Plasma	Expired air
$O_2 + HHb + KCl \rightarrow$ $\swarrow \quad \searrow$ $KHbO_2$	H ions \rightarrow Cl ions \rightarrow	$(H) + (Cl) + NaHCO_3 \rightarrow$ \searrow $NaCl$	$H_2CO_3 \rightarrow CO_2 + H_2O$
$HbNHCOOH + O_2 \rightarrow$ $\swarrow \quad \searrow$ HbO_2	H ions \rightarrow HCO_3	$H_2CO_3 \rightarrow$	$H_2O + CO_2$

6 Function of the Buffer Action—Since there is usually an excess of acid over base resulting from metabolism, it is obvious that the buffer action alone would not be sufficient to keep the pH constant. No matter how efficient the buffer solution, it does change slightly in pH with every addition of acid or alkali and if there is a constant excess of acid without any compensating mechanism, the blood would soon become neutral and the patient would die. The chief value of the buffers in the blood is to counteract the effects of sudden demands on the neutralizing ability of the body, till the acid or basic substance can be disposed of by other mechanisms. There must constantly be a good intake of basic substances in the food to restock the blood with neutralizing material.

B Excretion of Acid or (less commonly) Base—**1 As Carbon Dioxide by the Lungs**—The average daily excretion of carbon dioxide is equivalent to 20 to 40 liters of normal acid. This is by far the most important mechanism in maintaining the pH of the blood constant over long periods of time. If there is a tendency to acidosis, respiration is increased and carbon dioxide excretion increases. If there is a tendency to alkalosis respiration is slowed and carbon dioxide excretion decreases. This mechanism is seriously interfered with in extensive pulmonary disease such as pneumonia, depression of the respiratory center such as occurs in morphine poisoning, or in "oxygen hunger" such as occurs at high altitudes.

2 By the Kidney

(a) *Fixed acids* equivalent to 50 to 150 cc of normal acid are normally excreted daily in the urine. A good deal of this acid is in the form of salts. In ketosis the quantity is greatly increased.

(b) If excess of base is ingested, *alkali* (bicarbonate and alkaline phosphate) is excreted in the urine. The ability of the kidney to excrete excess of either acid or base is impaired in the conditions associated with nitrogen retention (chapter II).

C Conservation of Base—If all the acid to be neutralized were excreted in combination with base, the store of basic materials would soon be depleted. There are several mechanisms to prevent this.

1 Sulphuric acid is excreted in part as organic compounds, ethereal sulphates (e.g., indican), so that only one-half of the base otherwise needed is excreted.

2 Acetoacetic Acid—A large part is changed to the neutral substance acetone by loss of carbon dioxide (the carbon dioxide is eventually excreted by the lungs). The betahydroxybutyric acid formed from acetoacetic acid has, of course, the same acid value as the original acetoacetic.

3 Lactic Acid—All the lactic acid normally produced from carbohydrate during muscular activity or in the growth of cells is, if sufficient oxygen is present, either resynthesized to neutral carbohydrate or completely oxidized to carbon dioxide and water. When the oxygen supply does not keep pace with the amount of lactic acid produced, whether because of slowing of the circulation (as in cardiac failure or in anhydremia), or because of deficient oxygen per cc of blood (as in pulmonary disease or in anemia), or because of excessive lactic acid production (as after violent prolonged muscular exercise), the lactic acid is unchanged and accumulates in the blood and tissues, where it may cause

4 Excretion of an Acid Urine—The kidney has the unexplained selectivity to excrete acid sodium phosphate and the alkaline

when the alkali is needed. When the demand for alkali is great, the kidney is able to dissociate certain organic acids from their salts and excrete them in the free form. Among these are uric acid and hippuric acid. Hence, the greater the demand for alkali the more acid will be the reaction of the urine.

5 **Formation of Ammonia from Urea** (by the kidney) — This is said to occur in emergencies, the ammonia taking the place of basic radicals which remain in the blood stream while the ammonium salt of the acid is excreted. This must be distinguished from the increase in ammonia nitrogen in the urine which occurs in severe liver disease due to inability of the damaged organ to form urea.

Both mechanisms 4 and 5 may be seriously impaired in efficiency in those cases of kidney disease associated with nitrogen retention.

X ACIDOSIS AND ALKALOSIS

A Definition — 1 **Acidosis** is that condition of the body resulting from the presence in the blood and tissues of an amount of acid sufficient to lower either the alkali reserve of the blood or its pH, or both, below the normal limits.

Death occurs before the blood ever becomes acid, thus the term acidosis is a little confusing unless one remembers that it means simply a lessened degree of alkalinity.

2 **Alkalosis** is the presence in the blood and tissues of an amount of alkali sufficient to raise either the pH or the alkali reserve, or both, above the normal limits. It is not as frequent as acidosis, but should be recognized.

B Methods of Detection — All the methods discussed below are of some value in the detection of acidosis. The alkali reserve determination is the most valuable. The tests for alkalosis are the *alkali reserve estimation*, the pH of the blood, and the reaction and total acidity of the urine.

1 The alkali reserve estimation, also often called the carbon dioxide combining power of the plasma. This is much the most important test in disturbances of acid-base equilibrium. Its value has not been sufficiently recognized in the past, and, notwithstanding the simplicity of the technic of the titration method, it is not yet done nearly as often as it should be.

(a) *The indications* for doing an alkali reserve estimation are

(1) Kussmaul breathing (deep breathing without cyanosis)

(2) Coma, in practically every case

(3) Diabetes mellitus associated with the excretion of acetoacetic acid in the urine. The presence of acetoacetic acid in the urine is not alone a sufficient basis for the diagnosis of acidosis.

(4) Prolonged or severe vomiting.

(5) All conditions giving rise to a blood urea nitrogen of over 20 mg per 100 cc

(6) The toxemias of pregnancy

(7) During the prolonged administration of the following drugs sodium bicarbonate, magnesium oxide, calcium carbonate, mandelic acid, ammonium chloride, ammonium mandelate, or ammonium nitrate. Some believe it desirable to study the alkali reserve in patients receiving sulfanilamide. Patients with ulcer under medical management and patients to whom ammonium salts are being administered in large doses for their diuretic effect form most of this group. Since the maintenance of a moderate acidosis seems essential for the diuretic effect of the ammonium salts, it is only necessary to see that this acidosis does not become severe.

(8) Ketosis of severe enough grade to show a persistent, strongly positive acetoacetic acid test in the urine

(9) Gastric and duodenal fistula or continuous aspiration of the stomach

(10) Tetany

(11) Extensive burns

If an abnormal alkali reserve figure is found in the above conditions treatment should be instituted and alkali reserve estimations repeated at intervals of a few hours until they approach normal. As long as the above indications are present whether the alkali reserve has been previously normal or not, the estimations should be repeated at least once a week.

The results are expressed in terms of the alkali reserve figure which is the number of cc of dry carbon dioxide (measured at 0° C and 760 mm) which can be held in chemical combination, excluding dissolved carbon dioxide, by 100 cc of plasma after exposure to an atmosphere containing 5.5 per cent carbon dioxide (alveolar air) at 20° C.

(b) *Normal values* are 50 to 80 for adults and 40 to 60 for infants,¹ young children, and women during the last four months of pregnancy.

(c) *Interpretation of Pathologic Results*—Either acidosis or alkalosis may exist in the presence of a high, normal, or low alkali reserve figure. Theoretically, then, in order to state the true condition of acid base equilibrium present, it is necessary to know not only the alkali reserve figure, which indicates the bicarbonate level in the blood plasma, but also either the pH or the partial pressure of dissolved carbon dioxide, which indicates the carbonic acid level in the blood plasma. Practi

¹ Holman A. and Mathieu A. Blood Chemistry Studies of Normal Newborn Infants. II. Blood Sugar and Alkali Reserve Estimations. Am J Obst and Gynec 27: 95-98 (Jan) 1934. These authors have shown that the blood sugar and alkali reserve figure of the mother and infant are the same at birth.

cally, however, the only exceptions to the rules that a low alkali reserve figure indicates acidosis requiring treatment, and a high alkali reserve figure indicates alkalosis requiring treatment are a primary increase or decrease in the carbon dioxide tension in the blood

(1) Primary Decrease—This may be caused by hyperpnea not due to increased carbon dioxide or acid in the blood (e.g., the hyperpnea of high altitudes, fever, hot baths, voluntary hyperpnea, and that occurring as a form of hysteria or as a rare post lethargic encephalitis syndrome) Cyanosis does not occur In this form a “washing out” of carbon dioxide from the blood occurs which is compensated by excretion of bicarbonate with lowering of the alkali reserve Since this tends to keep the pH normal, it is desirable and alkalies are not indicated

(2) Primary Increase—Cyanosis is present in this group which includes all types of asphyxia, opium poisoning, deep anesthesia, the cyanosis of cardiac or pulmonary disease, etc This is compensated by a rise in the alkali reserve which tends to maintain the pH normal This rise is, therefore, desirable and does not require treatment

Both conditions are due to primary variations in dissolved carbon dioxide (H_2CO_3), tend to be temporary, are easily recognized clinically, and do not constitute indications for doing an alkali reserve estimation but rather for correction of the condition leading to the altered carbonic acid level of the blood

Practically, therefore, if the above exceptions are ruled out, alkali reserve estimations may be interpreted as follows

1. 40 to 50, mild acidosis with no clinical symptoms
 2. 30 to 40, moderate acidosis with definite symptoms
 3. 2 to 30, severe acidosis Patients are usually in coma or on the verge of coma Only immediate and efficient treatment will produce recovery if the figure is below 16

80 to 90, mild alkalosis usually not associated with symptoms

90 to 120, marked alkalosis usually associated with symptoms of tetany The causes of acidosis and alkalosis are discussed under C and D below

The carbon dioxide content or sodium bicarbonate concentration of the plasma is sometimes determined instead of the alkali reserve figure The results of the carbon dioxide content determination are expressed as the cc of dry carbon dioxide measured at 0° C and 760 mm which are held in chemical combination, excluding dissolved carbon dioxide by 100 cc of plasma of venous or arterial blood The normal values are 50 to 80 for venous and 40 to 55 for arterial plasma The corresponding values expressed as millimols of sodium bicarbonate per liter are 20 to 35 for venous plasma Since these determinations are much more difficult than the

titration method for carbon dioxide capacity and the interpretation is much the same, these determinations are chiefly of research interest (See Peters, J P, and Van Slyke D D Quantitative Clinical Chemistry Vol I and II The Williams and Wilkins Co Baltimore, 1931 for a detailed discussion)

2 The pH of the Blood—Accurate determinations require meticulous technic to prevent loss of carbon dioxide and expensive equipment and are not practical for clinical purposes Glass or quinhydrone electrode pH meters are necessary

(a) *Normals*—It remains quite constant at 7.3 to 7.5 Frequently the pH is normal when the patient has acidosis or alkalosis—See summaries of states of acid base equilibrium (X below)

(b) *Pathologic Results*—A pH of 7.0 to 7.3 indicates marked acidosis A pH of 7.5 to 7.8 indicates marked alkalosis Figures significantly below 7.0 or over 7.8 are incompatible with life Hence, even in the most marked acidosis the blood is not actually acid

3 Total Acidity and Reaction (pH) of the Urine—The reaction of the urine is usually included in a routine urinalysis although so many variables affect the pH that such a determination on a single sample is practically valueless To be of any value at all the reaction or the total acidity must be determined on a portion of the mixed 24 hour urine which has been collected under toluol The reaction depends largely on the relative proportions of acid forming and base forming materials ingested, but shows major daily fluctuations, such as the alkaline tide during digestion even in normal individuals When to these fluctuations are added the effects of drugs and in many instances, of decomposition changes occurring in the bladder or, more often from improper preservation after the urine is passed, and the fact that litmus paper gives only a very approximate idea of the pH the fallacy of depending on the reaction to litmus paper as ordinarily determined on a casually collected specimen becomes apparent Studies of the pH and total acidity are occasionally indicated in following the effects of dietary or drug treatment, or as a supplement to the alkali reserve estimation in acidosis or alkalosis The pH should be studied daily in patients under treatment for cystitis and pyelitis to make certain that the acidity or alkalinity desired is being attained

(a) *Normal values*—These are a total acidity of 150 to 400 cc of tenth normal sodium hydroxide to neutralize to phenolphthalein the entire 24 hour urine and a pH within the range of 5.1 to 7.0

(b) *Interpretation*—If no great preponderance of acid or base forming material is being ingested or injected a pH of less than 5.0 at all times of day or a total acidity of over 500 indicates a tendency to acidosis and a pH of over 7.0 at all times of day or a total acidity under 100 indicates a tendency to alkalosis if ammoniacal decomposition and impaired renal function are excluded Mandelic acid or methenamine is ineffective as a urinary antiseptic unless the urine pH can be maintained below 5.4¹ Sulfanilamide is more effective in controlling infections in the urinary tract if the urine is kept alkaline

4 Total Nitrogen and Ammonia Nitrogen Estimations in the Urine—This should be done on an accurately collected 24 hour sample of urine preserved with toluol The ammonia nitrogen should be calculated as per cent of the total nitrogen This is of some value in acidosis and in severe liver diseases

¹ Helmholtz H F The Effectiveness of Methenamine as a Urinary Antiseptic at Various Hydrogen Ion Concentrations J Ped 1 73-81 (July) 1932

See references to cystitis and pyelitis on page 49

The total nitrogen estimation on the 24 hour urine (plus 1 gm for the nitrogen excreted in the stool) multiplied by 6.25 gives the most accurate figure for the amount of protein being metabolized. This estimation is, therefore, essential for all accurate studies of protein metabolism, since part of the protein ingested may not be absorbed. Part of the nitrogen may come from body proteins that are being utilized or there may be nitrogen retained in new tissue that is being formed. The *nitrogen balance* is determined by comparing the nitrogen of the food (protein divided by 6.25) with the nitrogen excretion (total nitrogen of the urine plus 1 gm for the nitrogen in the feces). If these figures are approximately equal the individual is said to be in nitrogen equilibrium; if less nitrogen is excreted than is absorbed the balance is said to be positive, and if more nitrogen is excreted than is absorbed the balance is said to be negative. For persons who are expected to be in nitrogen equilibrium it is customary to calculate the protein of the diet by multiplying the nitrogen of the 24 hour urine plus 1.0 gm for the nitrogen of the feces by 6.25 which figure represents the actual grams of protein being metabolized.

(a) *Normal values*—About 0.5 gm of ammonia nitrogen is excreted per day and from 6 to 20 gm of total nitrogen depending on the diet. About 5 per cent of the total nitrogen is ammonia nitrogen. Over 10 per cent is definitely pathologic. Growing children and convalescents from wasting diseases should show a positive nitrogen balance; all others should be in nitrogen equilibrium.

(b) *Interpretation of Pathologic Results*—(1) Ammonia nitrogen constituting more than 10 per cent of the total nitrogen occurs in the following conditions:

(a) Severe cases of all types of acidosis except that due to impaired renal function.

(b) Severe diffuse liver damage such as occurs in eclampsia, acute yellow atrophy, phosphorous poisoning, etc.

(c) In ammoniacal decomposition of the urine such as occurs in some types of cystitis and pyelitis; these changed proportions will be found but always with an alkaline urine and pyuria. In groups (a) and (b) the urine will be acid.

Ammoniacal decomposition occurring after the urine is passed makes the results valueless and of course will not occur if the urine is collected under an efficient preservative.

(2) A negative nitrogen balance indicates a loss of tissue protein (muscle) and efforts should be made to increase protein absorption in such cases. The causes are:

First, a deficient protein intake or absorption as in starvation (stenosis of esophagus, vomiting, diarrhea, etc.) or improperly balanced diets (less than two thirds gram of protein per kilo in adults; less than one to three grams of protein per kilo in children, the requirement decreasing at periods of slow growth and increasing at periods of rapid growth).

Second, an excessive protein metabolism such as occurs in all the wasting diseases (tuberculosis, malignancy, prolonged fevers, etc.).

Third, in extensive muscular atrophies and in Simmonds disease (pituitary cachexia, a very rare condition).

5. *The Alkali Tolerance Test of Sellards*.¹—This is of some value in mild cases of acidosis where the patient is well enough to cooperate. It has the advantage that one is administering treatment at the same time that one is doing the test.

¹Palmer, W. W. and Van Slyke, D. D. Relationship between Alkali Retention and Alkali Excretion in Normal and Pathologic Individuals. *J. Biol. Chem.* 32: 477-50, (Dec.) 1917.

It is contraindicated in cases showing impaired renal function because of the danger of producing alkalosis before a change in pH occurs

(a) *Normals*—10 gm or less of sodium bicarbonate produces a change in pH

(b) *Interpretation of Pathologic Results*—A requirement of 12 to 30 gm indicates mild acidosis with no clinical symptoms

A requirement of over 30 gm indicates more severe acidosis with clinical symptoms

It is wiser, however, to use this test only in cases of mild or doubtful acidosis, and to stop giving alkali and to do an alkali reserve estimation if a distinct change in reaction does not occur after 10 grams of sodium bicarbonate have been administered

6 **Carbon Dioxide Tension of the Blood**—This may be determined directly on the venous or arterial blood by the point of junction for the figures of the carbon dioxide combining power and the pH in figure 2 or by the determination of the carbon dioxide tension of the alveolar air which is in equilibrium with arterial blood. While the total amount of carbon dioxide eliminated in a given time is much increased in acidosis the minute volume of air passing in and out of the lungs is so greatly increased that the actual percentage of carbon dioxide in the expired air is decreased. Marriott has devised a simple method which gives a roughly quantitative estimation of the percentage of carbon dioxide present. It is not nearly so reliable as the alkali reserve estimation. Results are expressed as partial pressure of carbon dioxide in mm of mercury. Strict normals are 40 to 45 mm. Results between 30 and 35 mm indicate mild acidosis, below 20 mm severe acidosis if respiratory stimulation of other types (caffeine, lowered oxygen tension in the inspired air, etc.) can be excluded. High values occur when the respiratory center is depressed (morphine poisoning, etc.) or any cause of carbon dioxide retention, such as drowning, tracheal occlusion, atelectasis pneumonia, etc. is present. A tension above 80 mm of mercury can persist only a very short time before death occurs.

The method can be used to advantage as a guide in regulating the carbon dioxide content of oxygen tents and chambers in the newer therapy of pneumonias,¹ by inserting a tube of the indicator buffer mixture in a shunt circuit for frequent or constant sampling of the gas mixture.

7 **Lactic Acid in the Blood**—The methods² are unfortunately too difficult for clinical use. Normal values are about 15 to 25 mg per 100 cc. Lactic acid estimations as high as 100 to 200 mg have been reported after severe muscular exercise and in lactic acid acidosis.

C **Causes of Acidosis**—1 **Severe and Prolonged Ketosis**—Diabetes mellitus and starvation are the most important causes of this type of acidosis, but any of the causes mentioned under ketosis may be

¹ Henderson Y, Haggard H W, Coryllos P N and Birnbaum G L. The Treatment of Pneumonia by Inhalation of Carbon Dioxide. Arch Int Med 45 72-91 (Jan) 1930

² Friedemann T E, Cotonio Magherita and Schaffer, P A. Acid. J Biol Chem 73 335 (May) 1927

Wendel W B. A Note on the Determination of lactic acid. J Biol Chem 102 47 (Sept) 1933

Ac J

responsible. This type of acidosis is usually recognized clinically. The laboratory findings are a low alkali reserve, acetoacetic acid in the urine and ammonia nitrogen over 10 per cent of the total nitrogen.

2 **Deficient Excretion of Normally Formed Acid**—(a) *The kidneys are unable to eliminate excess of acid radicals from metabolism of the food ingested*. Any type of impaired kidney function may lead to acidosis of this type. Ketosis is usually absent, and ammonia nitrogen is not increased in the urine. Many clinicians fail to realize the importance of always testing the alkali reserve in these cases and treating the acidosis when it is detected.

The laboratory findings are a blood urea nitrogen of 20 mg or over, and a low alkali reserve.

Impaired kidney function leads to difficulty in excreting either excess of acid or of base, hence the alkali reserve may be normal, increased, or decreased and shows no constant relation to the severity of the renal impairment. However the more severe the renal impairment the more frequently should the alkali reserve be determined, because slight changes in the ratio of acid to base forming materials taken in produce tremendous fluctuations in the alkali reserve. The author has seen a patient with severe renal damage due to mercury poisoning on the verge of death from acidosis three times and from alkalosis twice within a single week. When the acid and alkali intake were adjusted under the guidance of frequent alkali reserve estimations so as to keep the plasma bicarbonate level normal, this patient recovered completely, although she had taken many times the reputed lethal dose of mercury.

(b) *Asphyxia* from any cause (disease of lungs, tracheal stenosis, cardiac failure, etc). The acidosis is due to the retention of carbon dioxide as carbonic acid and to the deficient oxidation of lactic acid in the tissues. This disappears as soon as the cause is removed so that it is less important clinically than the other types. Morphine poisoning is a typical example of this type. Laboratory tests for acidosis are not indicated.

3 **Deficient oxidation of normally formed acid, chiefly lactic acid**. This may be due to

(a) *Slowed circulation* (as in dehydration, cardiac decompensation), or low blood volume after large acute hemorrhage.

(b) *Deficient aeration* of the blood in the lungs as in certain respiratory diseases.

(c) *Insufficient hemoglobin* to carry the necessary amount of oxygen as in anemias.

(d) *Excessive production of lactic acid* as after very violent muscular exercise.

Acidosis is of relatively minor importance in this group and disappears on treatment directed at the underlying cause. The conditions are easily recognized clinically and laboratory tests for acidosis are unnecessary.

4 Conditions in Which Increased Acid Production, Decreased Oxidation of Acid, and Decreased Elimination of Acid All Play a Part —(a) *Prolonged nausea and vomiting from any cause*, but especially the pernicious vomiting of pregnancy Ketosis and dehydration¹ are most important here There is also a tendency to alkalosis due to the loss of hydrochloric acid from the stomach so that an alkali reserve estimation should always be made High intestinal obstruction is especially apt to result in alkalosis

This group is very important as here several opposing factors operate Large fluctuations in the state of acid base equilibrium may occur in a short period of time The alkali reserve may be low, normal, or high irrespective of the severity of the underlying condition, but if not maintained as nearly normal as possible, acidosis or alkalosis may contribute to or cause a fatal outcome Other laboratory findings of importance are oliguria, ketosis, increased urea nitrogen in the blood low blood chlorides and increased ammonia nitrogen in the urine Intravenous or rectal administration of sufficient quantities of dextrose, sodium chloride, and water to maintain the above laboratory findings within normal limits produces striking clinical improvement

(b) *Severe diarrheas*, especially Asiatic cholera and the severe diarrheas of children Dehydration is the most important factor here, but loss of alkali through the bowel, ketosis from deficient carbohydrate absorption and toxic kidney injury also play a part

Oliguria and a low alkali reserve are the most important laboratory findings but urea nitrogen retention and ketosis may also occur Intravenous injection of fluid, dextrose and sodium bicarbonate (the latter must be sterilized in an autoclave or in an atmosphere of carbon dioxide to avoid changing it to carbonate) are indicated in sufficient dosage to keep the laboratory findings normal

In both (a) and (b) very frequent alkali reserve estimations are necessary to avoid over or under treatment

(c) In *pneumonia*, following *anesthesia*, and after *severe burns* of the skin an acidosis may develop due in part to dehydration and asphyxia, in part to ketosis and toxic kidney injury, but chiefly to the production of other acids which have not as yet been identified

5 Ingestion or Injection of Acid or Acid-producing Substances

(a) *Methyl Alcohol Poisoning* —Formic acid is produced in metabolism

(b) *Overdosage of Ammonium Chloride or Calcium Chloride* —A moderate acidosis is essential to the diuretic action of these drugs,

¹Marnett W M Anhydremia *Physiol Rev* 3 275-294 (April) 1923

but they should be temporarily discontinued if the alkali reserve figure goes below 30

(c) *Accidental or suicidal ingestion of strong acids* in considerable quantities

6 Cyclic Vomiting of Children—Ketosis is present and severe vomiting is the most outstanding clinical symptom. When this condition is suspected, the degree of acidosis should be determined as soon as possible by an alkali reserve estimation, then treatment should be started at once, as death may occur early. Roentgenographic study after a barium meal reveals peptic ulcer in many children with this syndrome.

It is possible that hypoglycemia¹ is a factor in this condition as well as in the pernicious vomiting of pregnancy. Therefore, blood sugar estimations should be done in such cases.

7 The Toxemias of Pregnancy (Chapter XI)—These may be associated with a marked acidosis requiring treatment. This must not be confused with the physiologic slight lowering of the alkali reserve which occurs in the last months of normal pregnancy and requires no treatment.

8 Anaphylactic Shock—This has been reported to give an acidosis with a lowered alkali reserve and to show clinical improvement on administration of alkali.

Note. Treatment of acidosis should be directed first toward removal of the cause, combined with ample fluid intake to prevent oliguria and a sufficient dextrose intake (with insulin if necessary) to prevent ketosis and to establish a glycogen reserve in the liver. If ketone bodies are not present or disappear from the urine and the alkali reserve estimation is still under 35 the administration of sodium bicarbonate, sodium citrate,² or sodium lactate³ will prove beneficial. This will only occasionally be necessary in diabetic acidosis, but treatment with alkalis is essential in the acidosis of impaired kidney function, and is desirable in most forms of acidosis not associated with ketosis. Calculate the dosage according to the following formula of

¹ Griffith J P C. Hypoglycemia and the Convulsions of Early Life. J A M A 93 1526-1529 (Nov 16) 1929

² Cape Jane and Sevringhaus E L. The Rate of Change of Alkali Reserve after Ingestion of Salts of Organic Compounds. II. Rate of Change of Alkali Reserve after Ingestion of Sodium Citrate and Sodium Bicarbonate. J Biol Chem 121 549-559 (Nov) 1937

³ Hartmann A F. Treatment of Severe Diabetic Acidosis. A Comparison of Methods with Particular Reference to the Use of Racemic Sodium Lactate. Arch Int Med 56 413-434 (Sept) 1935

Palmer and Van Slyke¹ as excessive dosage is apt to cause an alkalosis and has brought this therapy into disrepute

Rise in alkali reserve desired² times kilograms of body weight =
38

the correct dosage in grams of sodium bicarbonate

9 **Sulfanilamide**—In patients receiving large doses of sulfanilamide the alkali reserve is often low. Some³ believe this indicates an acidosis and recommend giving alkalies, but Hartmann, Perley and Barnett,⁴ with whom I agree, believe that this lowered alkali reserve is the result of excretion of base to compensate for an alkalosis of the primary carbon dioxide deficit type, resulting from hyperventilation produced by sulfanilamide.

D **Causes of Alkalosis**—1 Increased intake of alkali as food or drugs, or poisoning with strong alkalies.

This occurs most commonly in the medical treatment of peptic ulcer⁵ but is less apt to occur if insoluble alkalies such as calcium carbonate are substituted for sodium bicarbonate. It may also result from uncontrolled alkali treatment of acidosis or of nephritis, or from the Martin Fisher regime for overcoming edema, or in patients taking basic diets such as were advocated by Sansum and Blatherwick. In the author's experience all such therapy is safer for the patient and gives better clinical results if the alkali reserve figure is not allowed to exceed the normal limits.

Impaired renal function of all types showing nitrogen retention greatly predisposes to the development of alkalosis as well as of acidosis and the reaction of the urine is an unreliable criterion of the state of acid base balance in these cases. Hence, such cases should have very frequent alkali reserve estimations if alkali therapy is being given. Alkalosis itself may produce impaired renal function.

2 **Excessive Loss of Acid or Chloride from the Body**—(a) *Loss of Hydrochloric Acid*—This is a very important cause of alkalosis. It may be due to severe vomiting, particularly that of pyloric stenosis or

¹Footnote 1 p 81

²The alkali reserve figure desired minus the alkali reserve figure observed equals the rise in alkali reserve figure desired

³Long P H and Bliss Eleanor A. Toxic Manifestation of Sulfanilamide. Ann Surg 108 808-812 (Nov) 1938

Southworth H. Acidosis Associated with the Administration of Para amino benzene sulfonamide (Prontylin). Proc Soc Exper Biol & Med 36 58-61 (Feb) 1937

⁴Hartmann A F Perley A M and Barnett H L. Study of Some of the Physiological Effects of Sulfanilamide. Changes in Acid Base Balance. J Clin Investigation 17 65-472 (July) 1938

⁵Gatewood, W E et al. Alkalosis in Patients with Peptic Ulcer. Arch Int Med 42 105 (July) 1928

of high intestinal obstruction,¹ to gastric or duodenal fistula,² or profuse perspiration. Other chemical changes that occur are a decrease in the blood chlorides and an increase in the blood urea nitrogen. A marked ketosis from deficient carbohydrate intake may co exist with a severe alkalosis or may maintain the alkali reserve normal or produce an acidosis. If alkalosis is allowed to progress, the clinical syndrome of gastric tetany will occur (presumably due to a deficiency of calcium in ionic form although the total and diffusible calcium may be normal). Ammonium or sodium chloride should be given intravenously if the measures outlined above and by McVicar¹ do not promptly bring the alkali reserve within normal limits.

(b) *Excessive loss of carbonic acid as carbon dioxide* through the lungs. This may occur from voluntary forced respiration, from hysterical or post encephalitic hyperpnea, at high altitudes, during fever, or from hot baths. It is quickly compensated by decreased respiratory loss of carbon dioxide or by excretion of base. It is the only type of alkalosis which may coexist with a normal or low alkali reserve figure, but as these conditions are easily recognized clinically laboratory tests which might prove misleading are not indicated.

VI SUMMARY OF THE STATES OF ACID-BASE EQUILIBRIUM WHICH MAY OCCUR

In the following summary bicarbonate stands for the total alkali reserve, and H_2CO_3 for the acid substances (mainly carbon dioxide in solution and acid phosphate) in equilibrium with the basic material. N means normal. N+ means high normal. N- means low normal. X and Y refer to variable quantities of bicarbonate and carbonic acid. When the same letter occurs above and below the line, it means that the quantities of bicarbonate and carbonic acid, respectively indicated, are equivalent. In ratios 1 to 10 below, the number corresponds to the area having the same number in the accompanying chart (Fig. 2). Whenever there is a primary decrease or increase in either bicarbonate or carbonic acid this is compensated first by a change in the other factor sufficient to bring the pH within normal limits, the condition being represented by areas 2 or 3. Then there occurs a more gradual return to normal conditions represented by area 1, usually only after the cause of the initial deviation is corrected. Consequently, only very acute or very severe changes will produce a status of acid-base equilibrium not included within the lines representing pH 7.3 and 7.5.

$$1 \quad \frac{\text{Bicarbonate } N}{H_2CO_3 \quad N} = pH \text{ } N \quad \text{This is the normal condition}$$

$$2 \quad \frac{\text{Bicarbonate } N - X}{H_2CO_3 \quad N - X} = pH \text{ } N \quad \text{This usually indicates acidosis due to a}$$

primary lowering of bicarbonate as in ketosis or in impaired renal function. This is much the most common form of acidosis. It is treated by combating ketosis,

¹ McVicar, C. S. and Weir, J. F. Nature and Treatment of the Toxemia of Intestinal Obstruction and Ileus. J. A. M. A. 91: 887-901 (March 16) 1929.

² Walters, W., Kilgore, A. M. and Bollman, J. I. Changes in the Blood Resulting from Duodenal Fistula. J. A. M. A. 86: 186-187 (Jan. 16) 1926.

by increasing dextrose oxidation and by giving alkali. This is a *compensated acidosis* of the alkali deficit type, compensation is by blowing off of carbon dioxide. Deep breathing without cyanosis is the chief clinical symptom. The

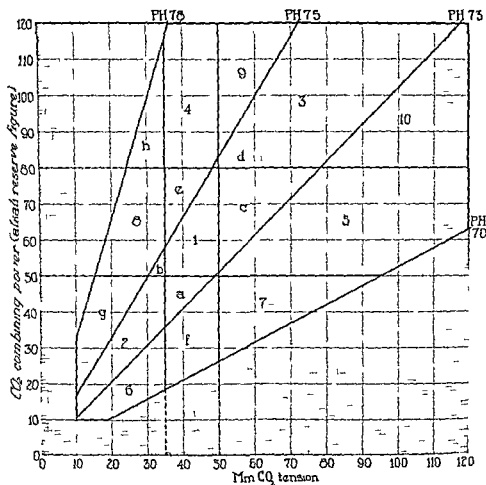


FIG. 2—Variations of pH, alkali reserve and CO_2 tension of blood¹

initial stage of this form of acidosis is indicated by the small area *a* expressed by the equation

$$\frac{\text{Bicarbonate } N - X}{\text{H}_2\text{CO}_3} = \text{pH } N -$$

The condition represented by area *2* may also result from alkalosis of the primary carbonic acid deficit type such as occurs from forced respirations. Alkali is excreted to compensate so that the lowered alkali reserve is desirable. Do not give alkali. This form is unimportant clinically. The initial phase of this condition is represented by the small area *b* expressed by the equation

$$\frac{\text{Bicarbonate } N -}{\text{H}_2\text{CO}_3} = \text{pH } N +$$

¹ The area outside of the heavy line representing a pH of 7.8, a carbon dioxide tension of 10, an alkali reserve of 10 and a pH of 7.0 represents states of acid base equilibrium which are incompatible with life. A carbon dioxide tension above 80 is incompatible with life if of more than a few minutes duration.

$$3 \quad \frac{\text{Bicarbonate } N + Y}{\text{H}_2\text{CO}_3 \quad N + Y} = \text{pH } N \quad \text{This condition usually results from a pri-}$$

mary increase in bicarbonate as after large dosage of alkalis, basic diets, or loss of hydrochloric acid. It is most apt to occur if impaired renal function co-exists. It is compensated by slowed respiration and retention of H_2CO_3 . No cyanosis occurs. This *compensated alkalosis* is the most common form. The initial stage is represented by the small area *d* expressed by the equation

$$\frac{\text{Bicarbonate } N + X}{\text{H}_2\text{CO}_3 \quad N + X} = \text{pH } N +$$

The condition represented by area 3 may also result from a primary increase in H_2CO_3 as in morphine poisoning, asphyxia, emphysema and other causes of cyanosis. This is the only condition in which the alkali reserve is increased in which treatment for alkalosis is not indicated. This condition results from compensation of an acidosis of the carbon dioxide retention type by selective retention of base and excretion of acid by the kidney. The condition in acute asphyxia is represented by the area *c* expressed by the equation

$$\frac{\text{Bicarbonate } N +}{\text{H}_2\text{CO}_3 \quad N + X} = \text{pH } N$$

$$4 \quad \frac{\text{Bicarbonate } N + Y}{\text{H}_2\text{CO}_3 \quad N} = \text{pH greater than } N \quad \text{Uncompensated alkalosis}$$

This is the first stage after ingestion of alkali. Some cases belonging to this group will fall in the area *e* expressed by the equation

$$\frac{\text{Bicarbonate } N +}{\text{H}_2\text{CO}_3 \quad N -} = \text{pH greater than } N$$

This compensates to 3 by slowed respiration and carbon dioxide retention. But if the excessive intake continues so rapidly that it cannot be compensated, this goes over into the fatal stage (9 page 90)

5 $\frac{\text{Bicarbonate } N}{\text{H}_2\text{CO}_3 \quad N + Y} = \text{pH less than } N$ *Temporary uncompensated acidosis*. This is the first stage of carbon dioxide retention as in therapeutic carbon dioxide administration, drowning, tracheal occlusion and other causes of acute cyanosis. Alkali is retained and it compensates to 3. This is the only form of acidosis in which the alkali reserve is normal and laboratory tests are not necessary for diagnosis.

6 $\frac{\text{Bicarbonate } N - X}{\text{H}_2\text{CO}_3 \quad N - X} = \text{pH less than } N$ This is the *fatal stage of uncompensated acidosis* (generally diabetic or nephritic). The body is unable to compensate the very low alkali reserve. The patient is usually in coma and will die if treatment is not immediately instituted. It is theoretically possible that a very few cases belonging to this group might develop very rapidly and have a complicating condition such as emphysema preventing the compensating loss of carbon dioxide. They would fall in the area *f* expressed by the equation

$$\frac{\text{Bicarbonate } N - X}{\text{H}_2\text{CO}_3 \quad N - X} = \text{pH less than } N$$

The prognosis in such cases would be exceedingly bad

7 $\frac{\text{Bicarbonate } N - X}{H_2CO_3, \quad N + Y} = \text{pH less than } N$ *Uncompensated acidosis* such as occurs in very deep anesthesia. These patients recover with artificial respiration, compensating to 1 or 2 by loss of carbon dioxide through the lungs and retention of base. Most of these cases probably pass through states of acid-base balance corresponding to areas c and 5 on going under the anesthetic and to f and a on coming out.

8 $\frac{\text{Bicarbonate } N}{H_2CO_3, \quad N - X} = \text{pH greater than } N$ This is *temporary uncompensated alkalosis* from hyperpnea as at high altitudes or from over-breathing. It compensates rapidly to 2 by excretion of base, and so is rarely found. Theoretically, forced hyperpnea such as occurs as a rare post-lethargic encephalitis syndrome if associated with renal impairment sufficient to prevent adequate compensatory excretion of base might give rise to the condition of uncompensated alkalosis represented by area g and expressed by the equation

$$\frac{\text{Bicarbonate } N - X}{H_2CO_3, \quad N - XY} = \text{pH greater than } N$$

This would be the only type of alkalosis in which the alkali reserve could be lower than normal. It is doubtful if it ever occurs. It is also theoretically possible that overdosage of alkali in a patient with the above mentioned syndrome might produce the condition corresponding to area h and expressed by the equation

$$\frac{\text{Bicarbonate } N + Y}{H_2CO_3, \quad N - X} = \text{pH greater than } N$$

For practical purposes this is so unlikely that it may be regarded as not occurring.

9 $\frac{\text{Bicarbonate } N + XY}{H_2CO_3, \quad N + X} = \text{pH greater than } N$ This is the *fatal stage of uncompensated alkalosis*. It should never occur if the administration of alkalis is controlled by alkali reserve estimations.

10 $\frac{\text{Bicarbonate } N + X}{H_2CO_3, \quad N + XY} = \text{pH less than } N$ This is *uncompensated acidosis* due to a sudden rise in H_2CO_3 without as yet a corresponding rise in bicarbonate. It is probably always temporary and due to acute severe asphyxia superimposed on a chronic asphyxia such as occurs when an acute pulmonary edema complicates an emphysema. It is a form of acidosis in which the alkali reserve is increased.

The vast majority of all patients studied will be found to belong in groups 1, 2, and 3, or their subdivisions.

Groups 4 to 7 are important but temporary conditions as they tend either to compensate or to lead to death.

Group 8 is unimportant, its subdivisions and groups 9 and 10 should never occur.

The exact condition of acid-base balance of any patient can be determined by reference to the chart, if one knows any two of the three variables: the alkali reserve estimation, the pH, and the carbon dioxide tension. The third variable may be determined from the chart as that point corresponding to the intersection of the coordinates of the two known factors. For clinical purposes

however, the alkali reserve estimation together with the results of a history and physical examination is satisfactory and more practical

VII MISCELLANEOUS TESTS OCCASIONALLY DONE IN DIABETES MELLITUS

A The D to N Ratio—For several days the patient is put on a diet consisting entirely of protein. The dextrose and nitrogen are estimated quantitatively in the 24 hour urine each day and the ratio of dextrose to nitrogen calculated. The results are used only when the ratio for two or three days in succession is constant. As the body is able to form about 58 gm. of dextrose from 100 gm. of protein and 100 gm. of protein is equivalent to 16 gm. of nitrogen (100 divided by 6.25) we know that a ratio of 3.65 to 1 (58 to 16) means that the patient is unable to oxidize any dextrose at all. Before insulin was available, any ratio over 3.4 to 1 was considered fatal. There is, of course, no normal ratio since all the dextrose is oxidized. The test was formerly used as an aid to prognosis but the determination of the patient's daily dextrose oxidizing ability or insulin coefficient gives much the same information in a safer way. The chief disadvantage of the D to N ratio determination is that the high protein diet is very bad for the patient.

B Diastatic Activity—This value, as measured by the quantity of starch which can be converted by the blood into dextrose in a given time, is somewhat increased in diabetes mellitus. It is not often determined at present as the blood sugar estimation and the dextrose tolerance curve are of greater diagnostic value. Very high or very low diastatic activity of the blood suggests disease of the pancreas but normal values are common even with extensive pancreatic disease.

C The Blood Fat and Cholesterol—These are often elevated in diabetes, sometimes resulting in a gross lipemia (turbid or even milky looking plasma). Quantitative estimations of these substances are interesting from an experimental standpoint, but are not necessary for diagnosis. The prognosis is said to be worse in cases with lipemia or hypercholesterolemia. (See blood cholesterol chapter II.)

XIII GRADES OF DIABETES MELLITUS

A Early or Incipient Diabetes—Occasional traces of dextrose occur in the urine. The fasting blood sugar varies from normal to 150 mg. The dextrose tolerance test shows a low diabetic curve.

B Mild—Dextrose and acetone are usually present in the urine. The fasting blood sugar is 150 to 250 mg. The alkali reserve is normal.

C Moderate—Dextrose, acetone, and acetoacetic acid are present in the urine. The fasting blood sugar is 250 to 400 mg. The alkali reserve figure is 40 or over.

D Severe—Much dextrose, acetone and acetoacetic acid are present in the urine. The fasting blood sugar is 400 to 1200 mg. per 100 cc. The alkali reserve figure is 30 to 40.

E Impending Coma—This demands immediate attention. It is characterized by acidosis. The alkali reserve figure is 10 or less. The blood sugar is 400 to 1200 mg. per 100 cc. are the same as in D. (See Chapter IV.)

These descriptions apply to untreated cases. The severity of treated cases is best determined by the dextrose oxidizing ability test or the insulin coefficient.

XIV LABORATORY CRITERIA FOR THE TREATMENT OF DIABETES MELLITUS

From a laboratory standpoint, if diabetes mellitus is to be regarded as controlled, the fasting blood sugar and alkali reserve figures must be within normal limits, and dextrose and acetone must be absent from the urine. In patients treated with protamine zinc insulin a one plus reduction in the morning urine is possibly desirable. If diabetic coma or impending coma is present, chief attention should be directed to control of the acidosis as evidenced by return of the alkali reserve figure to within normal limits. The ketosis should also be controlled as evidenced by the disappearance of acetone from the urine. Treatment with large amounts of dextrose and insulin should be continued not merely until consciousness has returned but until one feels sure that a good reserve of glycogen has been stored in the liver. During this period of treatment hyperglycemia and glycosuria may be neglected without great harm to the patient. After the initial blood sugar estimation to aid in establishing the diagnosis, blood sugar estimations need not be repeated until the above results are attained, if sufficient dextrose is given to maintain a constant glycosuria.

Hypoglycemia, not hyperglycemia, constitutes the danger during the period of active treatment of coma. Quantitative sugar estimations would have to be repeated so frequently to detect the rapid fluctuations in the blood sugar level due to the large doses of insulin and dextrose which are used that it is usually simpler and safer to test the urine for reduction every 15 to 60 minutes instead, and to increase the proportion of dextrose to insulin if reduction does not occur and decrease it if four plus reduction occurs.

In patients with diabetes mellitus who are not in immediate danger of coma, hyperglycemia and glycosuria should cause one to consider the advisability of reducing the carbohydrate intake, increasing the insulin dosage or, if glycosuria occurs only at one time of day as determined by the fractional reduction test, of readjusting the dosage of insulin or the relative proportions of carbohydrate in the meals. The latter procedure is usually more effective if protamine zinc insulin is being used. Acetone in the urine should cause one to consider the advisability of increasing the total dextrose value of the diet or of decreasing its ketogenic value. If dextrose and acetone are both present increase in insulin is

usually advisable first, then if the ketosis persists, it may be combated as above directed. A decrease in the alkali reserve figure is an indication for increase in the dextrose value of the diet with sufficient insulin to prevent glycosuria and hyperglycemia and if this acidosis persists after ketosis has disappeared (which is rare), an increase in the proportion of base-forming food in the diet or the actual administration of alkali is worthy of consideration.

The art and science of the treatment of diabetes mellitus is almost a specialty in itself and the few suggestions given above are but a part of it. The reason for their insertion is to suggest the help which laboratory data can give in therapeutics if one learns how to make use of them.

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CHAPTER IV

DISORDERS OF THE DUCTLESS GLANDS WITH ESPECIAL REFERENCE TO THE BASAL METABOLISM¹

I RESUMÉ OF THE ESSENTIAL POINTS IN THE PHYSIOLOGY, BIOCHEMISTRY² AND PATHOLOGY OF THE ENDOCRINE GLANDS AND THEIR RELATIONSHIP TO ENERGY METABOLISM

The energy of the body is derived from the oxidation of carbohydrates, proteins, fats, or of substances derived from these, such as amino acids, fatty acids, and lactic acid. Part of this energy is expended as work (muscular contraction), part in other ways, such as chemical changes, but as the laws³ of the conservation of matter and energy hold true for the living organism as well as for inanimate matter, it can all ultimately be accounted for, either as heat or potential energy. The most convenient measure for this energy production is the large calorie, which is defined as the amount of heat required to raise 1000 grams of water 1° Centigrade (from 15° to 16°).

Carbohydrate and fat are completely oxidized to carbon dioxide and water within the body under normal and most pathologic conditions. Hence, the energy derived and the end products produced are the same as those produced by the complete oxidation of these substances outside of the body, and may be expressed by the following equations

- 1 $C_6H_{12}O_6$ (Dextrose) + $6O_2 = 6H_2O + 6CO_2 + 40$ calories per
gram of dextrose, or 5.05 calories per liter of oxygen consumed
2 $2C_{57}H_{110}O_6$ (Tristearin) + $163O_2 = 110H_2O + 114CO_2 + 93$
calories per gram of fat, or 4.69 calories per liter of oxygen consumed

The end products of protein metabolism are, however, not only carbon dioxide and water but also nitrogenous substances, chiefly urea, which are excreted still incompletely oxidized. Hence, protein metabolism within the body consumes less oxygen and yields less energy than in the laboratory. The figures are 4.1 calories per gram of protein or 4.485 calories per liter of oxygen consumed. Certain of the amino acids from protein have a tendency to stimulate the metabolism, which is not yet fully explained but has been called *specific dynamic action*.

¹ DuBois E F. Basal Metabolism in Health and Disease. Ed 3. Pp 494. Lea and Febiger. Philadelphia 1936.

² Peters J P and Van Slyke D D. Quantitative Clinical Chemistry. Vol I. Pp 1-69. Williams and Wilkins Co. Baltimore 1931.

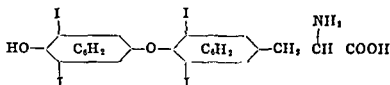
Thompson W O, Thompson Phebe K, Taylor S G, Nadler S B and Duchie Lois F N. The Pharmacology of the Thyroid in Man. J A M A 104 972-977 (Mar 23) 1935.

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³ These laws as well as the gas laws of Boyle and Gay Lussac and Avogadro's hypothesis should be reviewed in any text book on physics if they have been forgotten.

As a matter of fact energy never will be derived entirely from one food stuff. The simplest means of calculating the proportions of these different food stuffs actually being oxidized is the respiratory quotient. It is the volume of carbon dioxide given off, divided by the volume of oxygen absorbed (both measured at the same temperature and pressure) in a particular time interval. Knowing that equal numbers of molecules of gases occupy equal volumes under identical conditions of pressure and temperature, it is apparent from equation 1 above, that oxidation of carbohydrate alone would give a respiratory quotient of 1, and from equation 2 it is apparent that oxidation of fat alone would give a respiratory quotient of approximately 0.7. The oxidation of protein alone or of mixtures of these food stuffs would give intermediate respiratory quotients. The amount of energy produced (calories) per liter of oxygen consumed will vary with the substances being oxidized and, hence, with the respiratory quotient.

This oxidative metabolism must be maintained at a certain level to maintain health, even while at complete rest. Any increased demand for energy, such as muscular activity, must receive a prompt response. The regulation of the level of this oxidative metabolism seems to be chiefly by the hormone of the thyroid gland, although other substances influence it to some extent. The exact mechanism of the action of this hormone is not known, but it seems to be a catalyst. For our knowledge of the chemistry of this substance we are chiefly indebted to Kendall and Harington. A substance, thyroxin, which is very active in its effect on oxidative metabolism has been isolated and synthesized. Whether it is the hormone of the thyroid gland or an active group of a more complex compound is not yet settled. It is an iodine-containing compound with the following structural formula:



Thyroid hormone equivalent to about 15 mg. of this substance must be present in the body of an adult, outside the thyroid gland, to maintain oxidative metabolism at a normal level. There is evidence that it is excreted or destroyed at such a rate as to require an addition of hormone equivalent to 0.3 to 1.0 mg. of thyroxin per day to maintain this level.

A considerable decrease in the amount of thyroid hormone in the body in early childhood produces the syndrome known as cretinism, later it produces the syndrome known as myxedema. Slighter deficiencies produce less clear-cut changes. Increase in the amounts of the hormone produces one of the hyperthyroidism syndromes. That an iodine deficient or otherwise abnormal substance may be produced is a plausible but as yet unproved theory.

The concentration of thyroid hormone in the body outside the thyroid gland must be an algebraic summation of its rate of entrance (from the thyroid or by medication) and its rate of disappearance (destruction, excretion, storage?). The rate of formation of the hormone or its rate of entrance into the general circulation may be increased, decreased, or normal. Since this hormone may be stored in the colloid of the gland, the rate of formation and rate of entrance

into the circulation are not necessarily correlated. Metabolism determinations can hope to give only a clue to the concentration in the body outside the thyroid and the trend of that concentration. They do not tell whether the increase is due to rapid formation with a corresponding rate of entrance into the circulation, or to a rapid entrance into the circulation of previously accumulated stores. It is even theoretically possible that increase in concentration might be due to an abnormally slow rate of destruction or excretion, but there is no evidence suggesting that this occurs.

Conversely, a low concentration may be due to decreased entrance into the blood stream (not necessarily associated with decreased rate of formation) or to increased rate of destruction. There is good evidence that the former process is the more important, if not the only one which occurs.

In considering the pathologic background¹ for hypothyroidism or hyperthyroidism and the classification of goiter, the known and the hypothetical must be kept separate. It seems established that deficiency of thyroid parenchyma, whatever its cause, may result in the syndrome of hypothyroidism, and that hyperplasia of this parenchyma is often associated with the syndrome of hyperthyroidism. It seems possible that deficient function could arise from a deficient supply of the "building stones" (iodine, tyrosine?) for the thyroid hormone, or that systemic deficiency might be due to excessive storage of the hormone in the colloid of the thyroid. It has not been proved that the pathologic pictures usually classified as various types of goiter are anything but pictures of various functional states of the gland. Nor has it been proved that these functional states are due to a primary cause within the gland. There is evidence to suggest that unusual need for secretion, deficient or excessive supply of "building stones" for this secretion, and other factors outside the gland can profoundly modify its gross and microscopic appearance. It has not been proved that "adenomata" of the thyroid are neoplasms. Nor has it been demonstrated conclusively that when such nodules are associated with hyperthyroidism, the increase in thyroid hormone comes from the nodules and not from the thyroid parenchyma outside the nodules.

From this it is apparent that metabolic studies may give great aid in deciding as to the level of the thyroid hormone in the body outside the thyroid gland and thus aid in forming an opinion as to whether this gland is introducing a diminished (hypothyroidism), normal, or excess (hyperthyroidism) amount of the hormone into the blood stream, but that they will not tell one the nature of the pathology producing this change.

Hormones² of the pituitary also influence energy metabolism, but it is not yet known whether this influence is only indirect through thyrotropic hormones or is, in part, directly on the tissues. The pituitary influences carbohydrate metabolism as shown by alterations in the dextrose tolerance curve, variations in the ease of producing alimentary glycosuria, the disappearance of most of the

¹ Menne F. R. The Thyroid Gland in Hyperthyroidism. Arch. Path. 8: 954-975 (Dec.) 1929.

Rienhoff W. F. Jr. A New Conception of Some Morbid Changes Occurring in Diseases of the Thyroid Gland Based on Experimental Studies of the Normal Gland and the Thyroid in Exophthalmic Goiter. Medicine 10: 57-32 (Sept.) 1931.

² Russell Jane A. The Relation of the Anterior Pituitary to Carbohydrate Metabolism. Physiol. Rev. 18: 1-27 (Jan.) 1938.

signs of diabetes in pancreatectomized animals when the anterior lobe of the pituitary is removed, and the exaggeration of the symptoms of insulin deficiency by administration of anterior lobe extracts

To explain these effects a diabetogenic hormone of the anterior lobe of the pituitary has been postulated. Its mode of action is not yet known. Other hormones of the anterior pituitary include a gonadotropic hormone, a growth-stimulating hormone, a ketogenic hormone, a thyrotropic hormone, an adrenotropic hormone, a lactation stimulating hormone called prolactin related to the maternal instinct, and possibly others affecting fat metabolism. The middle lobe produces a hormone, intermedin, which passes through the posterior lobe and up the infundibular stalk to the region of the hypothalamus where it has a profound effect on water and salt excretion. Deficiency results in the syndrome of diabetes insipidus. A great variety of clinical syndromes may arise from many possible combinations of excess or deficiency of these various hormones and their effects on the other endocrine glands.

The adrenal produces epinephrin and cortin, epinephrin having an action similar to stimulation of the sympathetics in producing vasoconstriction and glycogenolysis. Cortin plays an important role in regulation of the level of sodium and potassium. The hormone of the parathyroids regulates the level of calcium. The ovarian hormones, theelin from the follicle and progestin from the corpus luteum, regulate the secondary sex characteristics, the menstrual cycle, and many of the uterine changes associated with pregnancy. The androgenic hormones from the interstitial cells of the testis regulate the male secondary sex characteristics, while the rate of formation of these hormones by the testes and ovaries are, in turn, regulated by the gonadotropic hormones from the pituitary. The placenta also forms an anterior pituitary-like gonadotropic hormone and a hormone, theelol, similar in structure and action to theelin. There is evidence that the thymus produces a hormone influencing the rate of reaching maturity but this effect becomes evident only after administration through several generations. Tests for all of the hormones are biologic rather than chemical. With the exception of the Friedman test for the anterior pituitary like gonadotropic hormone from the placenta, these tests are not practical for use by the average physician. Endocrinologists are beginning to use them in clinical medicine and it may not be long until practical tests for excess or deficiency of each hormone will be clinically available. The effects of excess or deficiency of insulin secreted by the islands of Langerhans of the pancreas have already been discussed.

II BASAL METABOLISM¹

The basal metabolic rate should be determined in every patient with suspected thyroid disease as it is not only the most valuable laboratory diagnostic aid in thyroid disease, but is also of value for prognosis, for following the course of such patients during treatment, and as a guide in selecting the best time for operation. It is also desirable to determine the metabolic rate in the thorough study of patients with pituitary dis-

¹ Boothby W. M. and Sandiford Irene. Basal Metabolism. *Physiol Rev* 4 69-161 (Jan) 1924

turbances, obesity, or leukemias and as a guide to therapy with thyroid preparations, dinitrophenol, or dinitro o cresol

A Definition—Metabolism in this connection includes only those metabolic processes that involve the using up of oxygen and the formation of carbon dioxide and water. Other types of metabolic processes such as reduction, hydrolysis, and synthesis are not gauged to any appreciable extent by the basal metabolic rate determination. The conditions are such that it represents the minimum heat production of which the patient is capable at the time of the experiment. Mental, physical, and digestive activity are prevented as far as possible, hence the use of the term *basal*. The basal metabolism of the patient, then, is the minimum oxidative or heat producing metabolism of which that patient is capable while awake and not under the influence of drugs.

B Principles Involved—The first experiments were done with calorimeters which measured the actual heat production of the body. This involved costly apparatus and the technic was too difficult for clinical use. However, by correlating the oxygen consumption, the carbon dioxide output, and the heat production of an individual, it was found that, knowing the oxygen consumption or the carbon dioxide output and the respiratory quotient, one could calculate very accurately the total heat production. All the methods that are clinically practical depend on this relatively simple procedure of estimating the oxygen consumption or the carbon dioxide output, or both, and calculating the metabolism in heat units from these results. By experiment it has been determined that the oxygen consumption methods give more reliable results than the carbon dioxide estimation methods. It has also been found that for practical purposes the respiratory quotient does not have to be determined on each patient but may be considered as the average figure of 0.82 under the conditions of the experiment. It is true that this figure will vary slightly in different individuals under the conditions of the test, but only in patients with diabetes has this variation been found to be sufficient to alter the results materially. Under basal conditions and with a respiratory quotient of 0.82, it has been further proved that the consumption of 1 liter of oxygen measured at 0° and 760 mm of mercury will produce 4.825 calories. Hence, knowing the total oxygen consumed in a given time, the total calories per hour may be easily calculated.

C Factors Influencing the Results—1 **Physiologic (a)—The Surface Area of the Patient**—There is much experimental evidence to show that in normal individuals of the same sex and age group the basal metabolic rate is more nearly a function of the surface area than

of any other body measurement. That is to say, a short thin man and a heavy man of the same age will have very different total caloric outputs in a given time, but if the calories per square meter of body surface are calculated for each the results will agree better than if, for instance, the calories per kilogram of body weight or per centimeter of height were calculated. Hence most basal metabolic rate standards are given in calories per square meter of body surface. Tables have been prepared from which the surface area can be read directly in square meters when the patient's height and weight are known. In the very obese and in patients with edema, there is a greater error in the surface area as determined from the tables than in persons of the average build. If it were possible to measure the correlation of total metabolism with the total mass of muscle and glandular tissue of the body would probably be the best.

(b) *Sex*—The basal metabolism in calories per square meter averages about 7 per cent higher in men than in women of the same age group. Before puberty, the sex difference is distinct but less marked.

(c) *Age*¹—After puberty, the metabolic rate in calories per square meter decreases at first rapidly and then more slowly throughout life so that it is about 30 per cent higher at 15 than it is at 75 years of age. The metabolism of children increases with growth in height and weight. It shows close correlation with either height, weight or surface area, corrected to age. The calories per square meter increase for the first 12 to 18 months of life, then decrease thereafter.

(d) *Race*²—Chinese, Japanese and other Orientals are said to have rates averaging 5 to 10 per cent lower than the Sage (Aub Du Bois) standards. The Mayas of Yucatan have a basal metabolism averaging about 8 per cent higher than the Harris and Benedict standards.

variations in total caloric requirement per 24 hours at different occupations. For example, while 2000 to 2500 calories may be a sufficient intake at sedentary occupations, 5000 or more calories may be required at such strenuous work as logging or harvesting. This increased oxygen consumption may persist for some time after the exertion is over. The explanation for this, as A. V. Hill has shown, is that substances produced by muscle contraction, chiefly lactic acid, may accumulate in considerable amounts and the oxidation of these substances may occur in part after, rather than during, the muscular contraction. This amount of oxygen needed to oxidize these residual metabolites has been called the oxygen debt. From this, one can see the importance of the period of rest and relaxation before the test if the true *basal* metabolism is to be determined.

Shivering, tremors, chorea, dyspnea, tics, or other muscular activity of any kind during the test will, of course, give rise to a rate which is higher than the true basal level.

Trained athletes and persons accustomed to severe muscular exertion show a true basal rate about 7 per cent higher than average healthy individuals otherwise comparable.

(f) *Dietary Habits*¹—Persons who habitually are on a vegetarian or greatly restricted diet gradually develop a lowered basal metabolic rate. The majority of such patients studied had rates of -5 to -20 per cent.

(g) *Digestion and Absorption*²—A light breakfast can increase the rate 7 per cent. A protein free meal may increase the rate 20 per cent, while a heavy protein meal, due to the specific dynamic action of the protein, may increase the rate 45 per cent. Absorption of liquids not requiring digestion, such as a dextrose solution, may produce a temporary increase in the rate of 5 per cent, and a cup of coffee may increase the rate 15 per cent. From these examples the importance of requiring a preliminary fasting period is obvious.

(h) *The Mental State of the Patient*—This makes a great difference. Worry, anxiety, nervousness, and mental activity increase the rate. This increase may persist for some hours after intense emotions. The ideal state for a determination is one of complete relaxation of mind as well as of body. Demonstrating the apparatus and explaining the

¹ Coons, Callie Mae and Schiefelbusch, Anna T. The Diets of College Women in Relation to Their Basal Metabolism. *J. Nutr.* 5: 459-465 (Sept.) 1932.

Wakeham, G. and Hansen, L. O. The Basal Metabolic Rates of Vegetarians. *J. Biol. Chem.* 97: 155-162 (July) 1932.

² Strang, J. M. and McClugage, H. B. The Specific Dynamic Action of Food in Abnormal States of Nutrition. *Am. J. Med. Sci.* 182: 49 (July) 1931.

harmless nature of the test may aid in allaying apprehension. Sleep, either natural or due to morphine, produces a lowered rate.

(i) *Menstruation*—There is normally a tendency to a slight premenstrual¹ rise and post menstrual fall in metabolic rate, but the specific effect is probably too slight to cause clinical error. Pain, discomfort, or changes in the emotional state are so commonly present, however, that it is better in most instances to defer estimations to the intermenstrual interval.

(j) *Pregnancy*—During normal pregnancy² the total metabolism increases to about 25 per cent above the non pregnant level at term and returns to its previous level by about the tenth day after confinement. The increase in weight is such that the basal metabolic rate as usually calculated shows an average increase of only about 10 per cent above the previous level and usually does not exceed plus 25 per cent, if reliable apparatus and technic are used. Almost all of this increase in metabolism occurs after the seventh month. It appears to be mainly due to the extra metabolism of the products of conception, chiefly the fetus itself, and not to any specific change in the maternal metabolism.

2 *Drugs and Tissues or Tissue Extracts*—(a) *Thyroxin and Related Substances*—These substances have by far the greatest and most prolonged effect. The average increase in metabolic rate in adults is about 2.8 per cent per mg. of thyroxin whether it is given at one time or in fractional daily doses. The maximum effect occurs about a week after administration and the effect of a single dose may persist for as long as forty days. It has been shown³ that the effect of thyroglobulin, thyroxin polypeptid, and desiccated thyroid on the basal metabolism is the same as that of an amount of thyroxin containing the same quantity of iodine. Since thyroxin contains about 65 per cent of iodine, the total iodine content of any thyroid preparation, divided by 0.65, will give the thyroxin equivalent of that preparation. Most desiccated thyroid preparations have an activity about $\frac{1}{250}$ of that of pure thyroxin. In other words, 1 mg. of thyroxin is equivalent to about 250 mg. or 4 grains of desiccated thyroid.

¹ Conklin, Claire J. and McClendon, J. F. The Basal Metabolic Rate in Relation to the Menstrual Cycle. *Arch. Int. Med.* 45: 125-135 (Jan.) 1930.

² Stander, H. J. and Peckham, C. H. Basal Metabolism in the Toxemias of Pregnancy. *Bull. Johns Hopkins Hosp.* 38: 227-236 (March) 1926.

³ Sandiford, Irene and Wheeler, Theodore. The Basal Metabolism before and after Pregnancy. *J. Biol. Chem.* 62: 329-352 (Dec.) 1924.

⁴ Lerman, J., and Salter, W. T. The Calorigenic Action of Thyroid and Some of Its Active Constituents. *Endocrinology* 18: 317-332 (May-June) 1934.

Thompson, W. O., McLellan, L. L., Thompson, Phebe K., and Dickie, Lois F. N. The Rates of Utilization of Thyroxine and of Desiccated Thyroid in Man. The Relation between the Iodine in Desiccated " " and in thyro " *Clin. Invest.* 12: 215-246 (Jan.) 1933.

(b) *Dinitrophenol*¹ and *Dinitro o cresol*—These drugs were introduced for the treatment of obesity but have been abandoned in scientific practice because of the danger of producing cataracts. Some proprietary reducing agents contain these drugs, however. They produce an immediate rise in the metabolic rate. After a single dose, the maximum effect occurs within 2 to 6 hours and disappears within 3 days. One mg per kilogram of body weight per day produces a maximum rise of plus 10 to plus 15 per cent in the metabolic rate which is reached in about 40 days and is maintained or decreased slightly if the same dosage is continued. Even after prolonged administration, the basal metabolic rate returns to its previous level within 3 days after the drug is discontinued. With marked overdosage, the metabolism is so greatly increased that heat production exceeds heat loss and fever results. The action of 4-6 dinitro o cresol is similar to the action of 2-4 dinitrophenol, but about 5 times as powerful. Neither drug is of value in the treatment of myxedema.

(c) *Caffein*—This causes a definite increase in rate persisting for several hours if given in large doses.

(d) *Epinephrin*—A marked but temporary rise is produced.

(e) *Pituitary Preparations* containing the thyrotropic hormone. These increase the rate by stimulation of thyroid activity.

(f) *Morphine and Dilaudid*²—These alkaloids produce a slight decrease in the rate, usually less than 10 per cent, similar to that which occurs from sleep or hypnotic suggestion of complete rest and relaxation.

(g) *Iodine*—This is usually without effect. However, it may cause certain cases of thyroid disease (previously without evidence of hyperthyroidism) to develop hyperthyroidism, and in the vast majority of instances causes a considerable fall in metabolic rate in cases of toxic hyperplastic (exophthalmic) goiter. This usually occurs quite suddenly about the eighth to tenth day after the administration is begun.

Other drugs and glandular extracts which are commonly used do not influence the basal metabolic rate sufficiently to interfere with the interpretation of the results.

¹ Cutting W C, Mehrtens H G and Tainter M L. Actions and Uses of Dinitrophenol. Promising Metabolic Applications. J A M A 101 193-195 (July 15) 1933.

Cutting W C and Tainter M L. Metabolic Actions of Dinitrophenol with the Use of Balanced and Unbalanced Diets. J A M A 101 2099-2102 (Dec 30) 1933.

Tainter M L, Stockton A B and Cutting W C. Use of Dinitrophenol in Obesity and Related Conditions. J A M A 101 1472 (Nov 4) 1933.

Rabinowitch I M and Fowler A F. Dinitrophenol. Canad Med Assn J 30 128-133 (Feb) 1934.

² David N A. Dilaudid and Morphine Effects on Basal Metabolism and Other Body Functions. J A M A 103 474-478 (Aug 18) 1934.

3 **Other Factors** —(a) *Fever* —A temperature elevation of 1 degree Fahrenheit increases the rate about 65 per cent. There is some effect if the patient has fever at any time of day even though the temperature is normal at the time of the test.

(b) *Chills or Shivering* —These produce an even more marked increase than fever.

(c) *Pain or Discomfort* —By causing mental activity and muscular tension these increase the rate. Therefore, it is important to make sure that the patient is comfortable during the test. A full bladder may be sufficient to cause an error in the result. Probably pain is a factor in some of the high estimations secured during menstruation.

D Preliminary Precautions and Directions to the Patient —The object of these is to eliminate as far as is possible all the factors mentioned above which influence the rate. The responsibility for these should rest with the physician himself, and not be left to technical assistants. *The reliability of the test depends to a large extent on the accuracy with which these details are attended to.*

1. Assure yourself the day before the test that the patient

(a) Has not received thyroid preparations¹ within a month and is not receiving dinitro o cresol, dinitrophenol, caffeine or epinephrin.

(b) Is not suffering from toothache, earache, headache, or any other cause of continued pain.

(c) Is not having fever or chills at any time of day.

(d) Is not menstruating, or due to menstruate within the next 48 hours.

2. Explain to the patient the harmless nature of the test and the importance of following instructions explicitly.

3. Write out² and give to the patient or his nurse the following instructions which the patient must follow to the letter.

(a) Eat a light evening meal about 6 P.M.

(b) Take nothing of any kind whatsoever after this meal until after the test is completed the next morning. If very thirsty, a little water at body temperature an hour or more before the test will do no harm, but milk or coffee or any other food or beverage must not be touched unless specifically permitted. A glass of orange juice several hours before the test will not introduce any material error.

(c) Spend the evening quietly at home, or preferably in the hospital, and go to bed by nine o'clock.

¹ The metabolic rate determination is of value in patients who are receiving thyroid or dinitrophenol preparations but it will not be a *basal* rate.

² Experience has demonstrated that oral instructions are usually misinterpreted or forgotten.

of white cells and the tissues producing them. A very high basal metabolic rate is a point in favor of acute rather than chronic leukemia.

Other abnormal conditions in which the determination is not indicated but the presence of which must be excluded or taken into consideration in interpreting high results include

(d) *Hypertension of Any Type* (most commonly hypertensive cardiovascular disease)—About 25 per cent of the cases not suffering from complications have rates above plus 10 per cent, and 10 per cent have rates above plus 15 per cent. Rates over plus 80 per cent have been reported. The majority of cases give results in the upper ranges of normal.

(e) *Perniciou anemia* and probably other severe anemias as well. Rates as high as plus 30 per cent have been reported. This is probably due largely to increased cardiac and respiratory effort to compensate for the diminished hemoglobin.

(f) *Fever* from any cause even when not present at the time of day the basal metabolism is determined.

(g) *Chills* from any cause.

(h) *Dyspnea* from any cause, hence most cases of cardiac decompensation.

(i) *Pain* from any cause, if at all severe.

(j) *Increased protein metabolism* from any cause.

(k) *Involuntary Muscular Movements of Any Type*—This group includes chorea, tics, convulsions, etc. I have seen two cases of Sydenham's chorea in which the clinical evidence of associated hyperthyroidism was very strong but the metabolic rate determination could not be used in the diagnoses because it was impossible to determine how much of the increase was due to the muscular contractions.

2 Causes of a Decreased Basal Metabolic Rate—Hypothyroidism is the most important.

(a) *Hypothyroidism*—This is the most valuable test we have in the diagnosis of myxedema. It is also low in untreated cretinism. Rates as low as minus 50 may occur. It is of great value in following the course of such cases under treatment and as a guide in estimating the dosage of thyroid substance to use. Since these preparations require a long time to take effect and the effect persists for weeks, the dosage should be gradually increased.

(b) *Hypopituitarism*—Low rates occur but are less constant than in the preceding group.

(c) *Addison's Disease*—A low rate occurs in some of these patients.

(d) *Obesity*, if due to endocrine deficiency. Other types of obesity have normal rates. Therefore the cases of obesity with low rates really belong under (a) or (b). It has been shown¹ that some people who have a marked tendency to obesity have a

¹ Mason, E. H. Obesity and Thinness. Studies on the Specific Dynamic Action in Them of Protein. Northwest Med. 26: 143-146 (March) 1927.

Goldzieher, M. A. and Gordon, M. B. Determination of the Specific Dynamic Action of Protein and Its Value in the Diagnosis of Pituitary Disease. Endocrinology 17: 569-577 (Sept.-Oct.) 1931.

very low specific dynamic response after eating protein, as compared to normal individuals. Use of thyroid preparations in treatment of obesity should be controlled by metabolic rate determinations.

(e) *Edema*—Severe edema with marked stretching of the skin may give rise to rates as low as minus 40 per cent. Low rates are especially characteristic of the edema of nephrosis and Epstein has reported benefit from thyroid therapy in this disease.

(f) *Undernutrition and emaciation* from any cause if there is no associated factor which tends to increase the rate. In this group are included patients with anorexia nervosa¹ malnutrition, inanition, asthenia, partial starvation and diabetes on undernutrition treatment. Most patients with diabetes have normal rates, but if they are on a low protein, low caloric diet the metabolism is low while on a high protein diet or if acidosis is present the metabolic rates may be above normal.

III OTHER LABORATORY TESTS OF VALUE IN ENDOCRINE DISORDERS

A *The Dextrose Tolerance Test*—This has been discussed in Chapter III. It is indicated in all patients with endocrine disturbances but it is more important in patients thought to have excessive or deficient function of the islands of Langerhans, the anterior lobe of the pituitary or the cortex of the adrenals.

B *The Blood Chloride Estimation*—This is discussed in Chapter V. It is indicated in patients thought to have Addison's disease both as an aid in diagnosis and in following the course of treatment.

C *Blood Calcium*²—This is of value in either hypo- or hyperfunction of the parathyroid glands. The calcium of the serum³ is about equally divided into diffusible and nondiffusible fractions. The nondiffusible fraction varies directly with the level of the serum proteins. Only the diffusible fraction is altered in the conditions in which a blood calcium estimation is indicated. A variable portion of this diffusible calcium exists in ionic form. The clinical pictures usually associated with alterations in blood calcium are due entirely to variations in this ionic form. Since there is no chemical method for determining the ionized calcium some have recommended estimating the diffusible fraction with the erroneous idea that this would give an indication of the ionized calcium. McLean and Hastings have devised a formula for calculation of ionic calcium from the serum protein and total protein estimations but this has been found unreliable⁴ in patients with marked alterations in the serum proteins and will probably prove unreliable in patients with alkalosis. Total serum calcium estimation is adequate for clinical purposes. If a diffusible calcium estimation is desired spinal fluid should be sent to the laboratory with the serum, since the spinal fluid constitutes an already prepared dialysate of the serum.

(a) *Indications*—A blood calcium estimation is indicated as an aid to diagnosis when osteomalacia, tetany (spasmophilia), rickets, Gee's disease, or parathyroid

¹ Farquharson R. F. and Hyland H. H. *Anorexia Nervosa A Metabolic Disorder of Psychologic Origin*. J. A. M. A. 111: 1085-1092 (Sept. 17) 1938.

² Peters J. I. and Van Slyke D. D. *Quantitative Clinical Chemistry*. Vol. I. pp. 805-861. Williams and Wilkins Co. Baltimore 1931.

³ Since calcium has been demonstrated in the preparation of heparin now available the use of heparin plasma for calcium estimation can no longer be recommended.

⁴ Gutman A. B. and Gutman Ethel B. *Relation of Serum Calcium to Serum Albumin and Globulins*. J. Clin. Investigation 16: 903-919 (Nov.) 1937.

disorder is suspected, and frequent estimations should be made during the course of these diseases as a guide to prognosis and further treatment. It is especially important to follow the blood calcium in patients who are receiving parathyroid preparations for it is possible so to raise the blood calcium by large doses of this substance that severe symptoms or death may result. Excessive doses of viosterol (irradiated ergosterol, Vitamin D) may also cause an increase in the calcium level.

(b) *Normal Figures* ¹—Figures for adults and children are the same, 9 to 12 mg per 100 cc of serum, or for the diffusible fraction 4 to 6 mg per 100 cc of spinal fluid.

The blood calcium is usually normal in cases of nonunion of fractures as the cause is usually mechanical rather than chemical. The calcium level in tuberculosis is normal and treatment to increase the calcium level has given disappointing results.

(c) *Diseases Associated with a Low Calcium Estimation*—(1) Osteomalacia

(2) Infantile tetany (spasmophilia)

(3) Rickets. This is much the most common cause in children, but the calcium level is frequently normal even in active rickets.

(4) Parathyroid tetany ². This is due to deficiency of the parathyroid hormone. A severe hypocalcemia with tetany not infrequently follows parathyroidectomy for hyperparathyroidism, so frequent serum calcium estimations are indicated in such cases.

(5) Gee's Disease (Idiopathic Steatorrhea or Nontropical Sprue) ³. This rare condition is characterized by diarrhea, fatty stools, tetany, osteomalacia, anemia, skin lesions, and infantilism.

(6) Impaired renal function. In all patients with impaired renal function sufficient to produce retention of phosphate, a low blood calcium may occur. This may be in part responsible for the irritation symptoms of uremia. In rare instances chronic impairment of kidney function persists long enough to permit the high phosphorus and low calcium to influence bone formation sufficiently to give the clinical picture of renal rickets or renal osteitis fibrosa cystica ⁴.

(7) Short intestine. In patients who have had a large part of the bowel removed, as for regional ileitis, severe calcium deficiency with tetany may result because of the insufficient length of intestine left for adequate absorption.

(8) Slight or inconstant lowering has been reported in pneumonia, hematogenous jaundice, dysentery, certain asthmas, sprue, furunculosis, and a small percentage of hemophilias.

(9) Hypoproteinemia. All of the causes given for hypoproteinemia may give a low serum calcium estimation due to depletion of the fraction combined with

¹ Schoenthal L, and Lurie Dorothy K. Concentration of Calcium and Phosphorus in the Serum of Children. *Am J Dis Child*, 46: 1038-1044 (Nov) 1933.

Molitch M, Weinstein S, and Cousins, R T. Serum Calcium in Normal Boys. *Am J Med Sci* 186: 378 (Sept) 1933.

² MacBryde C M. Treatment of Parathyroid Tetany with Dihydrotachysterol. *J A M A* 111: 304-307 (July 23) 1938.

³ Bennett T I, Hunter D, and Vaughan Janet M. Idiopathic Steatorrhea (Gee's Disease). A Nutritional Disturbance Associated with Tetany, Osteomalacia, and Anemia. *Quart J Med N S* 1: 603-677 (Oct) 1932.

Snell A M. Clinical Observations on Nontropical Sprue. *Arch Int Med* 57: 837-856 (May) 1936.

⁴ Albright F, Drake T G, and Sulkowitch H W. Renal Osteitis Fibrosa Cystica. Report of a Case with Discussion of Metabolic Aspects. *Bull Johns Hopkins Hosp* 60: 377-399 (June) 1937.

protein and not to any alteration in the diffusible fraction. These conditions do not constitute indications for doing a calcium estimation.

Calcium estimation is of value only in conditions (1) to (7), page 108.

Contrary to popular opinion the blood calcium is not low in hemorrhagic diseases and calcium therapy is of little, if any, value in the treatment of these conditions.

A deficiency of ionized calcium without alteration in total calcium occurs in severe alkalosis, giving rise to the clinical picture of tetany. The type which is due to excessive loss of hydrochloric acid from the stomach is sometimes called gastric tetany. An alkali reserve estimation is necessary for the diagnosis of this form of tetany.

(d) *Conditions Which May Give High Calcium Estimations*—(1) Hyperparathyroidism¹. This condition should be considered whenever multiple bone decalcifications, osteitis fibrosa cystica, or renal calculi² are noted. Renal calculi of calcium phosphate are common in patients with hyperparathyroidism but hyperparathyroidism is not a common cause of renal calculi.³ In hyperparathyroidism the blood calcium is usually between 11.5 and 19.0 mg per 100 cc of serum. Malignant tumors involving the bone, especially multiple myeloma, occasionally give a hypercalcemia, but the serum phosphorus in such patients is within normal limits which differentiates them from active hyperparathyroidism.

(2) Patients receiving active parathyroid preparations. The dosage should be controlled by blood calcium estimations since overdosage leads to hypercalcemia with attendant symptoms.

(3) Patients receiving overdoses of viosterol or dihydrotachysterol.

(4) Polycythemia vera.

(5) Myositis ossificans.

(6) Patients receiving calcium by mouth or intravenously. The increase is slight and temporary if parathormone, dihydrotachysterol, cod liver oil, or viosterol⁴ is not also given.

(7) Gout.

(8) Severe acne.

(9) Arthritis deformans.

(10) After injections of insulin a slight temporary increase occurs.

(11) Hyperproteinemia. In all of the conditions given as common causes of hyperproteinemia a high total calcium may occur without alteration in the diffusible calcium. A total protein estimation will sometimes be necessary in order to interpret properly the results of a total serum calcium estimation.

¹ Bulger, H. A. and Barr, D. P. The Relation of the Parathyroid Glands to Calcium Metabolism. *Ann Int Med* 5: 552-565 (Nov.) 1931.

Albright F., Aub, J. C. and Bauer, W. Hyperparathyroidism. A Common and Polymorphic Condition as Illustrated by Seventeen Proved Cases from One Clinic. *J A M A* 102: 1276-1287 (April 21) 1934.

Albright F., Sulkowitch, H. W. and Bloomberg, Esther. Hyperparathyroidism Due to Idiopathic Hypertrophy (Hyperplasia?) of Parathyroid Tissue. Follow up Report of Six Cases. *Arch Int Med* 62: 199-215 (Aug.) 1938.

² Albright F. Some Medical Aspects of Renal Stone Problem. *New England J Med* 217: 1063-1066 (Dec 30) 1937.

³ Griffin, M., Osterberg, A. E. and Braasch, W. F. Blood Calcium, Phosphorus and Phosphatase in Urinary Lithiasis. Parathyroid Disease as Etiologic Factor. *J A M A* 111: 683-685 (Aug 20) 1938.

⁴ Brougher, J. C. Viosterol (Irradiated Ergosterol) in Treatment of Parathyroid Tetany. *J A M A* 94: 471-473 (Feb 15) 1930.

LABORATORY DIAGNOSIS

Only in conditions (1) to (4) is the calcium estimation definitely indicated

D Inorganic Phosphate in the Blood—This test is indicated in the accurate study of hyperparathyroidism, bone disease and jaundice

(a) *Normal Figures*—The results are expressed in mg of phosphorus from inorganic phosphate in 100 cc of plasma In adults the normal range is 3 to 4 mg, in children it is 4 to 6 mg

(b) *Low figures* occur in active, untreated hyperparathyroidism rickets, osteomalacia, and after insulin injections

(c) *High figures* occur in patients with impaired renal function but the test is not necessary except in chronic cases when there is danger that renal rickets may develop In hyperparathyroidism the serum phosphorus may be high or normal in the inactive phase or following parathyroidectomy

E Serum Phosphatase—Several methods for the determination of the rate of hydrolysis of B glycerol phosphate when mixed with serum have been reported Results are recorded in units but at least two entirely different units have been suggested By the Bodansky technic the normal range in adults is 20 to 40 units per 100 cc of serum or plasma and in children 50 to 150 units Excessive formation or attempts at formation, of bone increases serum phosphatase This occurs in Paget's disease, osteomalacia, rickets, osteosclerosis, the osteoblastic type of osteogenic sarcoma and some cases of hyperparathyroidism A return to normal in the phosphatase level in these conditions indicates a tendency to healing High values also occur in obstructive jaundice¹ and liver disease Values of 10 units per 100 cc in a jaundiced patient suggest obstructive jaundice rather than a hemogenous or hepatic jaundice, but later studies² indicate that occasionally high values may result from diffuse liver disease The method is quite difficult and is rarely necessary for diagnosis

F Blood Cholesterol—This has been discussed in Chapter II Some have felt that blood cholesterol determinations should be done routinely in suspected thyroid disease since blood cholesterol values average high in hypothyroidism and average low in hyperthyroidism There is so much overlapping of the normal and there are so many other causes of alteration in the blood cholesterol level however that individual determinations have not proved of much assistance in diagnosis and are not nearly as dependable as the basal metabolism determination

G Blood Iodine Determination—This determination is of research value in

¹ Bodansky A and Jaffé H L Phosphatase Studies II Serum Phosphatase in Diseases of the Bone Interpretation and Significance Arch Int Med 54 88-110 (July) 1934

Bodansky A Determination of Serum Inorganic Phosphate and of Serum Phosphate Am J Clin Path (Tech Supp) 7 51-59 (Sept) 1937

Roe J H and Whitmore E R Clinico Pathologic Application of Serum Phosphatase Determinations with Special Reference to Lesions of the Bones Am J Clin Path 8 233-254 (May) 1938

Kellogg F S Smith J A Teel H M and Reid D E Recent Experience with a New Classification of Pregnancy Complicated by Hypertension and Albuminuria Am J Obst & Gynec 33 300-312 (Feb) 1937

Kay H D Phosphatase in Growth and Disease of Bone Physiol Rev 12 384-422 (July) 1932

² Rothman M M Meranze D R and Meranze T Blood Phosphatase As an Aid in the Differential Diagnosis of Jaundice Am J M Sc 192 526-535 (Oct) 1936

³ Freeman S Chen Y P and Ivy A C On the Cause of the Elevation of Serum Phosphatase in Jaundice J Biol Chem 124 79-87 (June) 1938

⁴ Davis C B Curtis G M and Cole Versa V Blood Iodine Studies II The Normal

thyroid disease but the technic is too difficult for practical clinical use except in the largest clinics. The normal level is 6 to 13 micrograms per 100 cc. It is usually elevated in patients with hyperthyroidism of less than 10 months duration but may be normal in persons with definite hyperthyroidism of longer duration.

H. Estimation of the Basal Metabolic Rate from Pulse Rate and Pulse Pressure¹ or Insensible Perspiration—A number of formulae have been devised for approximation of basal metabolic rate from the pulse rate and pulse pressure. The formula of Reid

$$BMR = 0.683 (PR + 0.9 PP) - 71.5$$

and of Gale and Gale

$$BMR = PR + PP - 111$$

have both been used. The latter is the simplest and is satisfactory to use as a rough check against gross error in performance of the metabolic rate determination but is not accurate enough to be of much clinical value alone.

Accurate metabolic rates may be calculated from the weight loss due to insensible perspiration but the expense of the sensitive large capacity balances required make this a research rather than a clinical method.

IV SUMMARY OF THE DIFFERENTIAL DIAGNOSIS OF ENDOCRINE DISTURBANCES

In all of these conditions the results of a physical examination give more information than does the laboratory examination.

A. Diseases of the Thyroid—1. **Diffuse Parenchymatous Hyperplasia (exophthalmic goiter)**—This should be thought of first if a basal metabolic rate above plus 30 per cent is found. Rates in the upper limits of normal may occur in remissions, but a normal rate is rare enough to constitute an indication for a reconsideration of the diagnosis. Rates as high as plus 110 per cent may occur. The determination should be repeated at least twice a week while following the course of a patient under medical treatment with rest, iodine, and diet. The best time for operation is as soon as the metabolic rate has ceased to fall. The level reached at this time is a valuable guide to the type of procedure indicated and the amount of tissue to remove. A subtotal thyroidectomy is not contraindicated by a rate of plus 30 per cent or less, but if medical measures do not bring it below plus 50 per cent, less serious procedures (ligation of vessels, roentgen therapy, etc.) are usually advisable as preliminaries to the thyroidectomy. In each case

¹Iodine Content of Human Blood. J. Lab. & Clin. Med. 19: 818-830 (May) 1934.

²Terkin H. J. and Lahey I. H. Exophthalmic Goiter: Relation between the Blood Iodine Level and the Duration of Symptoms in Three Hundred and Five Cases. Arch. Int. Med. 61: 83-89 (June) 1935.

³Corrooe B. I. Estimation of Basal Metabolic Rate from Pulse Rate and Pulse Pressure. Am. J. Med. Sc. 190: 31-376 (Sept.) 1935.

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must, of course, be judged for itself, taking into consideration all other factors as well as the metabolic rate. After operation, the rate should be determined at the time of discharge from the hospital and at intervals of 2 to 6 weeks thereafter, until it reaches a constant level. This serves to control the results of operation and to improve judgment in subsequent cases. A subnormal rate from post operative myxedema may indicate the desirability of giving thyroid preparations, or a persistently increased or rising rate may indicate the desirability of further therapy designed to reduce thyroid secretion before the patient's dissatisfaction with the operative result calls one of these sequelae more forcibly to the attention of a physician (usually not the one who performed the operation).

Other changes in laboratory findings which occur but are of little diagnostic value are the higher peak and longer time for return to normal of the dextrose tolerance curve with an increased tendency to alimentary glycosuria.

A normal erythrocyte sedimentation rate would aid in excluding infection as a cause for the symptoms but a rapid rate would not justify the opposite conclusion. Blood calcium estimations would aid in the early detection of post operative tetany and are necessary for the accurate control of the therapy designed to counteract this complication. The tendency to a lowered blood cholesterol¹ in hyperthyroidism may be of some diagnostic value.

The changes in laboratory findings in the vomiting or diarrhea of the acute crises are the same as in other types of severe vomiting or diarrhea and require the same therapy in addition to specific therapy with iodine.

2 Diffuse, Nodular, or Solitary Adenomatous Hyperplasia (adenomatous goiter)—This group can be separated from the preceding one only by the history and physical examination. Increase in the basal metabolic rate is on the average both less constant (less than 50 per cent of cases) and less marked but in an individual instance this is of little help. Otherwise, the laboratory study should be similar to that in the preceding group.

3 Myxedema and Cretinism—A truly basal metabolic rate of minus 10 or higher in a patient who had not received a thyroid preparation would exclude these diagnoses. Most cases under such conditions have rates of minus 20 or lower. The chief value of the basal metabolism determination in these cases is in the accurate control of the

¹ Hurxthal L. M. Blood Cholesterol in Thyroid Disease. I. Analysis of Findings in Toxic and in Nontoxic Goiter Before Treatment. Arch Int Med 51: 22-32 (Jan) 1933.
² Effect of Treatment. Arch Int Med 52: 86-95 (July) 1933.

dosage of thyroid preparations to maintain normal metabolism without producing hyperthyroidism. For this purpose metabolic rate determinations must be repeated at intervals of one to six weeks over a long period of time.

Normocytic anemia and decreased total blood volume are usually present and the hematology should be followed until it has returned to normal.

The low dextrose tolerance curve without glycosuria and the increased blood cholesterol¹ are of some diagnostic value.

The cause of the hypothyroidism must be discovered by other methods.

Patients with metabolic rates below minus 11, even without the clear cut myxedema syndrome, often derive benefit from properly controlled thyroid therapy.

4 Malignant Tumors—The diagnosis must be established by other than laboratory measures. The basal metabolic rate is not of differential value. It is most often elevated but may be normal or low. Anemia and increase in the erythrocyte sedimentation rate occur too late to be of aid in diagnosis.

5 Inflammations—In the acute stages the basal metabolic rate is usually elevated and the end result is often myxedema. Hence, when this diagnosis has been established, the metabolic rate should be periodically determined. The other laboratory findings are those characteristic of the type of infection present.

6 Colloid or Simple Goiter—These give rise to no changes in laboratory findings.

B The Pituitary²—Laboratory tests can give only evidence as to the function at the time of testing, not as to previous function nor as to the nature of the pathology giving rise to the altered function. They, alone, are rarely sufficient to differentiate hypo- or hyperfunction of the anterior lobe from corresponding changes in thyroid function. Because of the numerous hormones secreted by the pituitary it is rare to have a clinical syndrome due to excess or deficiency of only one. The differential diagnosis, therefore, is given in terms of the clinical syndromes rather than in terms of the hormones affected.

¹ Bronstein I. I. Studies in Cretinism and Hypothyroidism in Childhood. Blood Cholesterol. *J. A. M. A.* 100: 1661-1663 (May 27) 1933.

Hurxthal L. M. Blood Cholesterol and Thyroid Disease. III. Myxedema and Hypercholesterolemia. *Arch. Int. Med.* 53: 762 (May) 1934.

Hurxthal L. M. Blood Cholesterol and Hypometabolism. Suprarenal and Pituitary Deficiency (Obesity and Miscellaneous Conditions). *Arch. Int. Med.* 53: 825-831 (June) 1934.

² Kynearson L. H. and Hodgson C. H. Recent Advances in Knowledge of the Anterior Lobe of the Hypophysis. *Arch. Int. Med.* 62: 160-176 (July) 1938.

1 **Acromegaly and Gigantism**—An increase in the basal metabolic rate and a dextrose tolerance similar to that in hyperthyroidism with increased tendency to alimentary glycosuria constitute evidence of continued hypersecretion by the eosinophil cells of the anterior lobe, if other causes for these changes can be excluded. Many patients with the anatomical changes characteristic of acromegaly or gigantism will have basal metabolic rates and dextrose tolerance curves characteristic of normal or hypofunction. This is due to the fact that the initial stimulation may cease or may be followed by actual destruction of glandular tissue.

2 **Froehlich's Syndrome and Adult Pituitary Obesity**—During the stage of hypofunction the basal metabolism is lowered, the dextrose tolerance test gives a low peak and a quick return to normal and it is almost impossible to produce alimentary glycosuria. In these conditions, also, there is a tendency for the functional state of the gland to change while the anatomical features are still present. The site of the lesion and its nature must be established by other methods.

3 **Basophil Adenoma**—This is characterized clinically by hypertension, hirsutism, and a buffalo type of obesity with stria gravidarum. The renal function is not impaired and the dextrose tolerance curve may be high with an increased tendency to alimentary glycosuria. It must be differentiated from cortical adrenal tumors and arrhenoblastomas of the ovary by the history, physical findings and roentgenographic studies.

4 **Pituitary Dwarfism**—This is characterized by infantilism, both physical and sexual, without obesity. The basal metabolism and dextrose tolerance curves are low and alimentary glycosuria is difficult to produce.

5 **Pituitary Cachexia**—This is characterized by extreme emaciation, atrophy of all the organs, amenorrhea, loss of secondary sex characteristics, and decalcification of the bones with loss of teeth. The basal metabolism and dextrose tolerance curves are low. Alimentary glycosuria is difficult to produce. The body temperature is subnormal. It must be differentiated from anorexia nervosa and pancreatic disease by the history and necropsy findings. I¹ have seen a patient with all of the laboratory and clinical manifestations of pituitary cachexia in whom the pituitary was grossly and microscopically normal and no cause for the syndrome was found at necropsy.

6 **Diabetes Insipidus**—This is characterized by thirst and polyuria of 4 to 30 liters a day with urine of low specific gravity. It is

¹ Osgood E. E. Pituitary Cachexia? *Endocrinology* 23: 656-660 (Nov.) 1938

apparently due to deficiency of the hormones of the middle and posterior lobe or to lesions of the hypothalamus and pituitary stalk which prevent these hormones from reaching the hypothalamic region. It is controlled by intranasal insufflation of desiccated posterior pituitary.

Any of these pituitary syndromes constitute an indication for roentgenographic examination of the skull, neurologic examination, and examination of the cerebrospinal fluid for evidence of a tumor in the pituitary region. A craniopharyngioma will occasionally give rise to false positive serologic tests for syphilis in the cerebrospinal fluid.

C Suprarenals—Acute insufficiency is so rare and so unsatisfactorily studied from a laboratory standpoint that it will not be discussed.

1 Addison's Disease¹—This is due to cortical and usually also medullary deficiency. It is characterized by weakness, hypotension, and pigmentation of the skin and mucous membranes. The diagnosis is based on the physical examination. A low basal metabolic rate and low dextrose tolerance curve without glycosuria are confirmatory evidence. The blood chloride and urea nitrogen level will aid in judging the severity of the disease and the response to therapy with sodium salts, cortin, and low potassium diet. The lower the blood chloride and the higher the blood urea nitrogen the more severe is the deficiency of cortical hormone. A normal sedimentation rate would be one point against tuberculosis as the cause but would exclude neither it nor malignancy. Since amyloidosis is sometimes responsible for this syndrome, a congo red test would be indicated in the thorough study of such a case.

The vomiting and diarrhea which frequently occur, give rise to the usual changes in laboratory findings and these in turn have their usual indications for therapy.

Contrary to the original description of Addison anemia is not often present.

2 Adrenal Cortical Tumors—These are characterized by hirsutism and virilism and in children by precocious puberty. They must be differentiated from basophil adenoma of the pituitary and arrhenoblastoma of the ovary by the clinical findings and roentgenographic demonstration of the tumor after air injection.

D The Parathyroids—Parathyroid disease should be suspected if tetany or cystic bone lesions are found.

¹ Cutler H. H., Lower M. H. and Willey, J. A. M. A. 111: 117-122 (July 9) 1917.

Concentrations of Chloride, Sodium and Potassium in Urine and Blood. Their Diagnostic Significance in Adrenal Insufficiency.

1 **Hypoparathyroidism** —This is characterized by the symptoms of tetany with a low blood calcium due to decrease in the diffusible calcium

2 **Hyperparathyroidism** —This is characterized by osteitis fibrosa cystica and sometimes calcium phosphate renal calculi. The blood calcium is elevated and the inorganic phosphate is decreased during the active phase. Urinary excretion of calcium may be greatly increased. The serum phosphatase is usually elevated.

E The Gonads —Hypofunction or hyperfunction of the ovaries or testes must be recognized by clinical methods although biologic tests for excess or deficiency of these hormones are available for research studies.¹

F The Polyglandular Syndromes —There are many such syndromes but the differential diagnosis of the type and degree of glandular involvement must be made largely by clinical methods. A low basal metabolic rate and dextrose tolerance curve and normocytic anemia may occur.

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¹ Gallagher T F, Peterson D H, Dorfman R I, Kenyon A T and Koch F C The Daily Urinary Excretion of Estrogenic and Androgenic Substances by Normal Men and Women. *J Clin Investigation* 16 695-703 (Sept) 1937

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CHAPTER V

DISORDERS OF THE GASTROINTESTINAL TRACT¹

I DISORDERS OF THE STOMACH

A Resume of the Essential Points in the Physiology² and Biochemistry

1 The Amount and Character of the Contents of the Stomach at Any Given Time —This is a resultant of the algebraic summation of the amount and character of any of the following additions and subtractions, which may have occurred since the stomach was last empty

(a) *The Secretions of the Gastric Mucosa* —These include gastric juice and mucus

(b) *Abnormal Substances* which may have been added from the stomach wall These include transudates, exudates of various types, or blood itself

(c) *Additions through the Esophagus or by Stomach Tube* —These include

(1) Substances of any type which may have been ingested or given by stomach tube

(2) Exudates, transudates, blood, or secretions which may have been swallowed coming from the lungs, bronchi, trachea, mouth, nasopharynx, sinuses or esophagus

(3) Contents of esophageal diverticuli or material retained above an esophageal stenosis or cardiospasm, etc

(d) *Substances Which May Have Entered through the Pylorus* —These may include bile, duodenal secretions, pancreatic juice, or intestinal contents, rarely even fecal matter from the large bowel, or blood, organisms (bacteria, animal parasites), exudates or transudates, etc, from these sources

(e) *Substances Which May Have Entered by the Following Pathologic Routes*—(1) Fistulae to the exterior (2) Fistulae to other hollow viscera, i e, gastroenterostomy, gastrojejunal or gastrocolic fistulae (intestinal contents, etc), fistulae to the gall bladder (bile, gallstones, etc)

(f) *The Amount and Character of All Subtractions* that have taken place since the stomach was last empty These may occur by any of the routes above

¹ Rehfuess M E Diseases of the Digestive Tract and Allied Organs the Liver Pancreas and Peritoneum Progressive Medicine 4 2-133 (Dec) 1929

Best C H and Taylor N B The Physiologic Basis of Medical Practice Pp 672-750 William Wood & Company, Baltimore 1937

² Ivy A C The Newer Physiology of the Gastrointestinal Tract Am J Med Sc 173 453-460 (April) 1927

Ivy A C Contributions to the Physiology of the Stomach IX The Causes of Gastric Secretion Their Practical Significance and the Mechanisms Concerned J A M A, 85 877-880 (Sept 19) 1925

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mentioned, but passage from the stomach on into the intestinal tract, and passage out through the esophagus (vomiting, withdrawal through the stomach tube) are the most important, although some substances (alcohol) are absorbed by the gastric mucosa, and stomach contents may pass out through a perforation into the peritoneal cavity or through fistulous tracts into other organs

Since most of these additions and subtractions are individually variable both in amount and in character and the character may be altered by their interactions with each other (this interaction may be chemical, bacterial, or enzymatic) it is obvious that as many of these variables as possible must be controlled, and that even then the interpretation of alterations in the composition of stomach contents must be made with great caution

Furthermore, as most of these additions and subtractions occur gradually and at more or less localized points, and as the stomach is a relatively inefficient mixing machine when active and subject to numerous influences both internal and external which inhibit this motor activity entirely, it is unsafe to assume that any fraction of its total content will correctly represent the composition of the remainder of that content. Experimental evidence¹ confirms this reasoning

2 The Composition and Secretion of the Gastric Juice —The most important constituents of pure human gastric juice are water, hydrochloric acid, the enzyme pepsin, and the intrinsic factor of Castle which reacts with the extrinsic factor in food to form the antipernicious anemia principle. Of less importance are inorganic salts, chiefly chloride, and the enzymes rennin and gastric lipase. The exact site and mechanism of this secretion are still disputed. The concentration of hydrochloric acid is relatively constant at 0.4 to 0.5 per cent, equivalent to 110 to 140 cc of N/10 acid per 100 cc of pure juice. This concentration may diminish but does not increase even in disease, the lower values usually found in stomach contents being due to dilution, combination (with protein substances, e.g., food or mucin), and neutralization effects

All that is definitely known as to the mechanism of the formation of this strong mineral acid is that it is derived from chlorides of the blood either by the retention or more probably by the reabsorption of base. The chlorine content of the secretion remains relatively constant in health and disease, the changes in acidity being roughly in inverse ratio to the amount of basic radicals present. During this acid secretion the corresponding amount of base set free tends to increase the alkali reserve figure in the blood and to decrease the acidity of the urine (alkaline tide) while at the same time there is a decrease in the chlorides in the blood. The presence or absence of this alkaline tide has even been suggested as a criterion for judging the secretion of acid by the stomach, but the urine reaction is subject to too many other variables for this to prove very reliable

The pepsin is secreted in the form of pepsinogen which is inactive, requiring the presence of a sufficient hydrogen ion concentration (free acidity) to convert it into the active proteolytic enzyme. This enzyme in turn is active only in acid solution and shows its optimum activity at a pH of 1.4 to 1.6 corresponding to

¹Gorham F. D. The Factor of Dilution in Gastric Analysis. *J. A. M. A.* 81: 1738-1742 (Nov. 24) 1923

Gorham F. D. Variations of Acid Concentration in Different Portions of the Gastric Chyme and its Relation to Clinical Methods of Gastric Analysis. *Arch. Int. Med.* 2: 434-440 (April) 1921

removal of the fasting contents may be of diagnostic as well as therapeutic value

C Planning the Examination—Laboratory diagnosis should be individualized just as much as therapeutics should be. Each type of test will detect certain alterations in gastric pathology, physiology and chemistry and miss others, while all of them suffer from the serious limitations of our inability to control most of the numerous extragastric variables. Therefore, from a consideration of the points mentioned under A and B above, it is obvious that time spent in debate over the relative merits of this or that test meal, or over this or that time and manner of securing stomach contents could better be spent in planning the particular tests to carry out on the individual patient to secure the information one desires with the least loss of time, discomfort, and expense to that patient.

At best we can only hope to get approximate information on the following points: (1) the ability of the stomach to secrete hydrochloric acid and enzymes with or without unusual stimulation, (2) the time required for the stomach to empty itself with a greater or lesser load, (3) the presence or absence of pathologic substances (exudates, blood, bacteria, parasites, lactic acid) but no proof that their source is in the stomach, (4) some idea of gastric digestion (under definitely abnormal conditions and confused by some admixture with other enzymes) of the substances introduced into the stomach since it was last empty.

D Choosing the Test Meal—For the majority of cases I recommend the modified Ewald meal as the initial test, to be followed by the histamine test if no free hydrochloric acid is found, and such other tests from the group below as seem best suited to give the further information desired. In most cases no other test will be found necessary.

Much more accurate results will be secured if the stomach is emptied of its fasting contents before a meal is given. Often examination of the fasting contents alone will give all the information desired, since it is the best procedure for detecting severe grades of retention, abnormal exudates, blood, micro organisms and lactic acid, although the other tests will usually give this information.

The Ewald meal or one of its modifications will yield the most information in the simplest way if the object desired is to test the ability of the stomach to secrete gastric juice under a normal stimulus, and to digest a simple meal.

¹ Gaither E. H. Diagnostic Value of Secretory Function in Gastric Disease. Various Methods Studied and Compared. *Ann Int Med* 5: 992-996 (Feb.) 1932.

The fractional method will yield the most information if the object is to study the physiology of the normal stomach or to detect slight deviations from the normal, provided the subject is first so habituated to the stomach tube that its presence does not cause distress. However, it is also the most troublesome to perform and in my opinion the additional information obtained rarely justifies its use.

The histamine test is best (but is not without danger) if it is desired to study as nearly pure gastric juice as is possible, or the ability of the stomach to secrete free hydrochloric acid under a maximum stimulus.

A large dinner of the Riegel type is most satisfactory if one wishes to detect slight degrees of impairment of gastric function, but this may do harm if given to patients with greater degrees of impaired function.

The stasis meal will best detect moderate or slight degrees of retention.

The use of 49 cc. of seven per cent alcohol to which has been added 1 cc. of 0.1 per cent "phenolphthalein" (phenolsulphonphthalein?) in 95 per cent alcohol has been advocated as a substitute for the usual test meals with the claim that by colorimetric determination of the percentage of the dye in the stomach contents withdrawn, the amount of dilution of the gastric juice could be calculated and therefore the actual volume and acidity of the pure gastric juice. This would be true if neither the dye nor the alcohol were selectively absorbed by the stomach wall, if the meal were the only factor tending to dilute the gastric juice and if no substances (saliva, mucus exudate, blood, duodenal contents) capable of combining with hydrochloric acid had entered the stomach. The re-emphasis of the error due to dilution by the meal was desirable, but the quantitative estimation will probably prove more misleading than helpful.

It should not be necessary to mention that every record of stomach contents analysis should clearly state whether the stomach was emptied before giving the test meal, what was the character and quantity of the test meal, what was the time interval between the beginning of the test and the withdrawal of the stomach contents, and whether all or only a part was withdrawn. When no test meal has been given, the record should state whether the specimen analyzed is fasting contents, vomitus, or a specimen secured for a check on the treatment, in all of which cases the composition of the last meal and the interval since it was ingested and the character of the last therapy and the interval since it was given should be recorded.

E Interpretation of the Results of Stomach Contents Examination¹—1. Ewald Meal and Its Modifications —(a) *Normals*²—The volume varies from 30 to 70 cc. but only great deviations from this are

¹ Crohn B. B. *Affections of the Stomach*. Pp. 100-155. Saunders Philadelphia 1927.

² Vanzant F. R., Alvarez W. C., Eusterman G. B., Dunn H. L. and Berkson, J. The Normal Range of Gastric Acidity from Youth to Old Age. An Analysis of 3746 Records. *Arch. Int. Med.* 49: 345-359 (March) 1932.

significant It separates into two layers on standing, the lower containing partially digested food, the upper being relatively clear Small amounts of bile and mucus are often grossly visible It is usually acid (blue) to the congo red paper test *Free hydrochloric acid* is usually present in quantities corresponding to 15 to 75 cc of N/10 acid per 100 cc The *total acidity* is usually equivalent to 30 to 90 cc of N/10 acid per 100 cc The figures for women average about 10 points lower than for men About 4 per cent of children otherwise normal show no free hydrochloric acid (achlorhydria) and this percentage increases with age to about 30 per cent of persons in the seventh decade *Pepsin* is present as indicated by digestion of the albumin in Mette's tubes The *lactic acid* test is negative, and *blood* is usually absent, but small amounts due to trauma from the tube are not rare

Microscopic examination shows *food residues* only from the test meal and these undergoing digestion *Sarcinae* or *Boas Oppler bacilli* are usually said to be absent Actually they have been repeatedly demonstrated by culture,¹ and a few organisms may occasionally be found in the stained smears even in stomach contents showing normal acidity and peptic activity Other bacteria and yeasts may also be present The bactericidal and bacteriostatic activity of normal gastric contents is usually overemphasized Further work on the flora of the normal stomach is urgently needed

In children, the total volume is, of course, smaller, and the free and total acidity are lower In the first five years of life the free acidity averages 15, gradually increasing to an average of about 30 at puberty and reaching the adult average of about 45 for men and 35 for women at the age of twenty Other findings are similar to those in adults

(b) *Increased Volume*—Only volumes of 120 cc or over are significant The increase may be due to increased secretion, decreased emptying or both The causes are

(1) Obstruction at or near the pylorus This may be due to ulcer of the stomach or duodenum, to carcinoma or less commonly to benign tumors of the stomach, to congenital stenosis of the pylorus, to high intestinal obstruction, or rarely to syphilis of the stomach

(2) Acute or chronic atonic dilatation of the stomach The acute form (suspected if marked distention and tympany of the upper abdomen associated with evidences of cardiac and respiratory embarrassment are present) is an often overlooked indication for use of the stomach tube It may occur as a postoperative complication, or produce a sudden change for the worse in a great variety of illnesses The chronic

¹ Burget G E Note on the Flora of the Stomach J Bact 5 299-303 (May) 1920

form occurs in association with ptosis, after habitual over filling (beer drinker's stomach) or without clear etiology

(c) *Decreased Volume*—Volumes below 20 cc (when not due to incomplete emptying as might occur when the stomach tube does not extend into the stomach contents fully or when it becomes occluded with particles) may result from decreased secretion, rapid emptying (most important) or both, or from failure to pass the tube into the distal segment of an hour glass stomach. The causes are

(1) Pernicious anemia and other cases with achylia (due to diminished secretion)

(2) "Leather bottle stomach" The stomach is very small, the wall is rigid and it empties very rapidly. Most of these cases are due to *scirrhus carcinoma*, and a few to *syphilis of the stomach*. *Linitis plastica* is reputed to produce this same picture, but this diagnosis should rarely if ever be made since most cases so diagnosed prove to be *scirrhus carcinoma* rather than chronic infection.

(3) *Hypermotility* This may occur reflexly from a large number of causes, chief of which are peptic ulcer and gall bladder disease.

(4) *Abnormally large exit*, the so called "dumping stomach" This occurs when too large an opening is left after a gastroenterostomy or pyloroplasty and in some cases when fistulae develop between the stomach and bowel.

(5) *External pressure* This may be due to ascites or large intra abdominal tumors (especially to enlarged spleen).

(6) *Hour-glass stomach* This is most often due to peptic ulcer, but may be due to syphilis or carcinoma. The volume may be normal if the stomach tube finds its way through the opening into the distal pouch.

(7) *Large benign tumors or foreign body accumulations* such as hairball or phytobezoar. Think of phytobezoar in persons who have been indulging freely in persimmons. These are rare causes.

Fluoroscopic examination after a barium meal is usually necessary to differentiate these conditions.

(d) *Excess of Mucus*—This occurs in passive congestion of the stomach, inflammations of the stomach wall such as gastritis, or phlegmon, chemical irritation from alcohol or highly spiced foods in some cases of ulcer or carcinoma, and as a result of nausea. Presence of the stomach tube may produce enough nausea to influence the acidity by excessive mucus production in fractional tests. If much is present one must consider its neutralizing effect when interpreting the acidity figures.

(e) *Achlorhydria*¹—This is the absence of free hydrochloric acid. If gastric enzymes are also absent (no digestion of egg white in Mett's tubes) it is called *achylia*². Topfer's reagent is not sufficiently specific for free hydrochloric acid to be relied on, hence, if the clinical figure for free acidity is fifteen or lower with this reagent, a qualitative test with Boas' reagent should be done also. Only when this test is negative can we conclude that there is achlorhydria.

The finding of achlorhydria after an Ewald meal should be confirmed, if possible, by the histamine test, since most modifying conditions tend to decrease the free hydrochloric acid. A positive Boas test on one occasion is more significant than many previous negative tests.

The causes of achlorhydria are

(1) Pernicious anemia (achylia). A diagnosis of pernicious anemia is usually incorrect if free hydrochloric acid is found. Achylia precedes the onset of the anemia by a considerable period. The gastric contents are usually alkaline.³

(2) Carcinoma of the stomach.⁴ Achlorhydria is the rule in advanced cases, but in early cases (when the diagnosis should be made) hypochlorhydria or normal acidity is often found. Lowered acidity is a point in favor of carcinoma as compared to ulcer but no type of figure will exclude either diagnosis.

(3) Syphilis of the stomach. This is relatively uncommon, but most of these patients show achlorhydria. This diagnosis should be considered if a patient clinically suspected of having peptic ulcer shows achlorhydria.

¹ Bockus H L, Bank J and Willard J H. Achlorhydria. With a Review of 210 Cases in Patients with Gastrointestinal Complaints. *Am J Med Sci* 184: 185-201 (Aug) 1932.

² Accurate quantitative methods for the determination of pepsin in gastric contents have been developed but the technic is so difficult and the information obtained of such slight diagnostic value that their use is of research rather than clinical interest.

Osterberg A E, Vanzant Frances R and Alvarez W C. Studies of Gastric Pepsin I. Methods of Measurement and Factors Which Influence It. *J Clin Invest* 12: 551-556 (May) 1933.

Vanzant Frances R, Osterberg A E, Alvarez W C and Rivers, A B. Studies of Gastric Pepsin II. Secretion of Pepsin in Cases of Duodenal Ulcer and Pseudo Ulcer. *J Clin Invest* 12: 557-565 (May) 1933.

Vanzant Frances R, Osterberg A E, Alvarez W C, Judd E S and Rivers A B. Studies of Pepsin in Human Gastric Juice V. Its Prognostic Value. *Am J Digest Dis & Nutrition* 3: 101-102 (Apr) 1936.

Anson M L and Mirsky A E. The Estimation of Pepsin with Hemoglobin. *J Gen Physiol* 16: 59-63 (Sept) 1932.

³ Helmer O M and Fouts P J. Gastric Analysis Methods. *Am J Clin Path (Tech Supp)* 7: 41-50 (Sept) 1937.

⁴ Comfort M W and Vanzant Frances R. Gastric Acidity in Carcinoma of the Stomach. *Am J Surg* 26: 447-456 (Dec) 1934.

(4) Chronic atrophic gastritis Achylia occurs in the late stages of chronic gastritis This diagnosis should not be made until the three conditions above listed have been excluded

(5) Idiopathic hypochromic anemia¹ This is the name given to an anemia with low color, volume and saturation indexes occurring most commonly in middle aged women and due in all probability to deficient absorption of iron secondary to poor gastric digestion of the organic iron compounds present in foods Achlorhydria is present in about fifty per cent of the cases Three to six grams per day of ferric ammonium citrate or ferric carbonate results in a prompt return of the blood picture to normal without any effect on the achlorhydria

The above are the only groups showing achlorhydria in more than 50 per cent of cases A greater incidence of achlorhydria than in symptom free persons of the same age groups occurs in hyperthyroidism, hypothyroidism, chronic infectious arthritis, combined system disease without anemia, pellagra, pernicious vomiting of pregnancy, etc, but is of little or no diagnostic value Achlorhydria or achylia is apparently sometimes responsible for a chronic diarrhea, but it will be recalled that 5 to 30 per cent (probably fewer if the histamine test had been used) of adults show achlorhydria without symptoms These patients should be watched more closely than others for signs of anemia and combined system disease

(f) *Hypochlorhydria*—This is a diminished free hydrochloric acid It may be due to deficient secretion (low total acidity also), or to combination with protein (total acidity higher), or to neutralization (total acidity low, normal, or high) The causes are the same as for achlorhydria (with the exception of pernicious anemia) but it is much less significant Hypochlorhydria is common during normal pregnancy² with a return to normal following delivery

(g) *Hyperchlorhydria*³—This is an increase in the free and total acid of the stomach contents It was formerly thought to be due to the secretion of a more acid gastric juice but it is now known that the normal gastric juice has a maximum acid content, and that clinical hyperchlorhydria is due to an increased quantity of secretion or to lessened

¹Hartfall S J and Witts L J Gastric Secretion in Simple Achlorhydric and Allied Anaemias and the Intrinsic Factor of Castle in Simple Achlorhydric Anaemia Guy's Hospital Reports 83 3-36 (Jan) 1933

²Strauss M B and Castle W B Studies of Anemia in Pregnancy I Gastric Secretion in Pregnancy and the Puerperium Am J Med Sci 184 655 (Nov) 1932

³Vanzant F R Alvarez W C Berkson J and Fusterman G B Changes in Gastric Acidity in Peptic Ulcer Cholecystitis and Other Diseases Analyzed with Help of New and Accurate Technic. Arch Int Med 52 616-631 (Oct) 1933

neutralization or to both factors. It occurs most frequently in peptic ulcer of the duodenum, stomach or esophagus, but the acids may be normal, diminished, or absent in the presence of ulcer. Furthermore, hyperchlorhydria occurs in other conditions. It is of little diagnostic value but is a point in favor of peptic ulcer when the differential diagnosis from syphilis or cancer arises.

(h) *The Total and Combined Acidity*—The total acidity tends to vary with the free hydrochloric acid. The combined acidity (difference between the two) may be increased due to excessive neutralization of hydrochloric acid or due to the production of organic acids by fermentation. The latter occurs only when the free hydrochloric acid is low. Therefore, when the free hydrochloric acid figure is normal or high the total acid figure represents the actual acid due to gastric juice (if acids have not been ingested) more accurately than does the free acidity, while either may be the more accurate if the free acid is low.

(i) *The pH*—This is decreased (1.4 or less) in the conditions listed as causing hyperchlorhydria and increased (2.0 or over) in those causing achlorhydria or hypochlorhydria. It is not a satisfactory substitute for the titration methods.

(j) *Lactic Acid*—This should be tested for if the free hydrochloric figure is less than 30. Its presence is due to a sufficient stasis and decrease in acidity to permit fermentation. It is, therefore, found in many cases of carcinoma, but may occur in any other condition giving rise to stasis with hypochlorhydria. Since a small amount of lactic acid is present in certain foods, only a distinct test is significant.

(k) *Blood*¹—Small amounts, particularly if still red, may be due to trauma from the stomach tube. Blood which has been in contact with acid for more than a few moments is changed to acid hematin (brownish). Considerable amounts of changed blood give the appearance of coffee grounds. The causes of blood in the stomach contents are:

(1) Peptic ulcer. Ulcer of the duodenum, the stomach or esophagus, or near the orifice of a gastroenterostomy is the most common cause in persons under 40 years of age.

(2) Carcinoma of the stomach or esophagus. This is the most common cause in persons over 40 years of age.

(3) Varices in the lower end of the esophagus or cardiac end of the stomach. These result from obstruction to the portal or splenic veins.

¹ Bortz, E. L. Diffuse Hemorrhage from the Stomach. *Arch. Int. Med.* 50: 1-26 (July) 1932.
Rivers, A. B., and Wilbur, D. L. Intrinsic Gastroduodenal Lesions as Causative Factors of Hematemesis. *Arch. Int. Med.* 50: 621-634 (Oct.) 1932.

such as occurs in cirrhosis of the liver, Banti's disease, or thromboses of these veins. Large hemorrhages occurring intermittently are characteristic of this condition.

(4) Benign tumors. These are rare.

(5) Chronic passive congestion.

(6) Violent or prolonged vomiting from any cause.

(7) Agonal erosions of the gastric mucosa. These occur in moribund patients.

(8) Swallowed blood. This may be from a cracked nipple of the mother in infants, from the mouth if there are bleeding gums or ulcers, from the nasopharynx, from the lungs, trachea or bronchi in any of the conditions listed as causes of hemoptysis, or in all types of ulcerations of the esophagus including peptic ulcer, carcinoma of the esophagus or lung, ruptured mediastinal lymph node, or leaking aortic aneurysm.

(9) Hemorrhagic diseases.

(10) Diffuse hemorrhage or hemorrhage from erosions. This is commonest between the ages of 20 and 40 and occurs in cases of diaphragmatic hernia and in persons who are apparently perfectly well. It may complicate a great variety of diseases due to infections, cardiovascular pathology, or chemical poisons. The diagnosis must be made by exclusion of the other causes.

The causes listed under (5) to (10) inclusive are often overlooked in attempting to interpret the presence of blood in the gastric contents. The absence of blood does not exclude any of the diagnoses listed. A positive blood test will, of course, be secured if meat is present in the stomach contents.

(1) *Food Residues from a Previous Meal*—Identification of such residues indicates stasis. This may be due to

(1) Impaired motility. This occurs in acute or chronic atonic dilatation of the stomach.

(2) Obstruction at or near the pylorus. Stasis results only after decompensation has occurred. Peptic ulcer of the stomach or duodenum, active or healed, is the commonest cause in adults, but it may be due to carcinoma, to benign tumors, to spasm of the pyloric ring, or secondary to high intestinal obstruction. In infants it is often a result of congenital pyloric stenosis.

(m) *Boas Oppler Bacilli*—These are significant only if they are present in large enough numbers to be found in almost every field on the slide. They may occur in any condition producing stasis with diminished acidity and are most common in carcinoma producing pyloric obstruction.

(n) *Sarcinae*—These are, also, significant only if present in large numbers. They indicate stasis without decrease in acidity. Hence, they are most common in ulcers producing pyloric obstruction and their presence is a point against, but does not exclude, the diagnosis of carcinoma of the stomach.

(o) *Tubercle Bacilli*¹—An examination of the fasting stomach contents for tubercle bacilli by the concentration technic is indicated when pulmonary tuberculosis is suspected and sputum examinations are negative. This is especially valuable in children.

(p) *Pus*—This is rarely found in stomach contents. If grossly purulent stomach contents are found in a patient complaining of severe epigastric pain, phlegmonous gastritis should be considered as a possibility. A source in the mouth or respiratory tract must be excluded.

2 **Fasting Stomach Contents**—The interpretation is the same as for contents taken after the Ewald meal with the exceptions that absence of acid is somewhat less significant and increase in volume is more significant, while food residues from a previous meal and tubercle bacilli are more easily detected.

3 **Vomitus**—Valuable information is often lost by failure to examine vomitus. Such an examination may obviate the necessity of a test meal. Volume variations and food residues must be interpreted in relation to the volume of the last meal and the time since it was ingested. Lactic acid and blood are of little significance unless present in large amounts. Hematemesis may result from any of the causes listed for blood in gastric contents after an Ewald meal but occult blood may be due to meat, and even gross hemorrhages may be due to violent vomiting alone.² If there is any question of poisoning it is important to save the vomitus or fasting stomach contents in a sealed container in a refrigerator for toxicologic examination.

4 **The Histamine Test**³—This should be done as the initial if pernicious anemia is suspected and as a control on any patient showing achlorhydria or hypochlorhydria to the Ewald test meal. It is superior to the Ewald meal for determination of gastric ability to secrete acid and inferior for other purposes. The normal clinical figures for maximum total acidity (the free hydrochloric acid is about 10 points less than the total acidity so that it need not be estimated) which occurs in the 10 minute interval between 20 minutes and 30 minutes after the histamine injection are 50 to 150 cc. and the normal volume of secretion is 5 to 60 cc. for this 10 minute interval. Achlorhydria or hypochlorhydria after this powerful stimulus is much more significant than after an Ewald meal. The interpretation of abnormal results is the same as is given for the Ewald meal. Only about 10 per cent of normal persons show achlorhydria with this technic.

¹ Ulmar, D. and Ornstein, G. G. Gastric Examination in Pulmonary Tuberculosis with Negative Sputum. *J. A. M. A.* 101: 835-836 (Sept. 9) 1933.

² Mallory, G. K. and Weiss, S. Hemorrhages from Lacerations of the Cardiac Orifice of the Stomach Due to Vomiting. *Am. J. Med. Sc.* 178: 506-515 (Oct.) 1929.

³ Pollard, W. S. Histamine Test Meals: An Analysis of Nine Hundred and Eighty Eight Consecutive Tests. *Arch. Int. Med.* 51: 903-919 (June) 1933.

Babkin, B. P. Some Recent Advances in the Physiology of Gastric Secretion. *Am. J. Digest. Dis.* 5: 107-112 (Apr.) 1938.

Martin's¹ studies suggest that a nonprotein nitrogen or a urea nitrogen on the gastric contents secreted after histamine stimulation is of value in the diagnosis of carcinoma of the stomach. Normal values are for nonprotein nitrogen 20 to 30 mg per 100 cc, for urea nitrogen 13 to 40 mg per 100 cc. In carcinoma values of 63 to 124 mg and of 6 to 3 mg were obtained for nonprotein and urea nitrogen respectively. Lesser increases occurred in gastric or duodenal ulcers, benign achlorhydria and pernicious anemia.

5 **The Stasis Meal**—This should be given when impaired motility or obstruction to the outlet is suspected and has not been demonstrated by the Ewald meal or by fluoroscopy. The fluoroscopic examination after a barium meal has largely supplanted this test.

6 **The Riegel Meal or a Meal of Its Type**—These large meals are the best test for slight degrees of impairment of total gastric function and are not used nearly enough. Such a meal should not be given until severe obstruction or impaired motility has been eliminated by an Ewald meal or fluoroscopic examination or both. Most information is secured if a small sample is removed at three hours for tests of acidity and observation of the progress of digestion and if the stomach is emptied at six or seven hours to note whether any food residues remain. The subjective sensations of the patient are also worthy of observation as distress typical of gall bladder disease, ulcer or irritable colon may be brought on by such a meal. The meat of course nullifies the value of an occult blood test. Acid and enzymes should be present in good concentration and digestion well advanced at three hours. At six or seven hours the stomach should contain less than 100 cc of fluid of a consistency resembling puree. Only wide deviations from these findings are significant.

7 **The Fractional Test Meal**²—Some prefer this to the Ewald meal for routine use. It is somewhat more apt to detect free hydrochloric acid than is the Ewald meal and much less apt than is the histamine test. In my opinion it is inferior to the Ewald meal supplemented by the histamine test for clinical use. It is much more trouble to do and the curves add little to the diagnostic significance at best and are sometimes misleading.

8 **The Alcohol Test Meals**—These give no information on gastric digestion or delayed emptying but are satisfactory as a preliminary to the histamine test if the ability of the stomach to secrete hydrochloric acid is all that is desired. I prefer the Ewald meal supplemented by the histamine test.

9 **Stomach Contents Removed as a Control on Ulcer Therapy**—It is especially important that the record of such an examination show the time of obtaining the material (usually 4:30 or 9:30 P.M.) and the purpose of the examination. Since the object of most ulcer therapy is to prevent peptic digestion at the site of the ulcer by neutralizing hydrochloric acid with protein or alkalies as fast as it is formed, thus preventing the change of pepsinogen to pepsin, it is important that the test for free hydrochloric acid should be negative. If free hydrochloric acid is present the advisability of increasing the dose of alkali or protein food or of altering the time of their administration should be considered. Increased volume especially in the evening aspiration suggests obstruction at the pylorus or hypersecretion.

¹ Martin L. Protein Nitrogen and Nonprotein Nitrogen Determinations on Gastric Juice. A Clinical Evaluation. J. A. M. A. 100: 1475-1478 (May 13) 1933.
² Bockus H. L., Glassmire C. and Bank, J. Fractional Gastric Analysis in 200 Cases of Duodenal Ulcer. Am. J. Surg. 12: 6-1 (April) 1931.

Blood, not due to trauma from the stomach tube, which is found more than ten days after adequate therapy has been started in a case thought to be gastric ulcer, should lead one to consider the possibility of carcinoma

Γ Blood Chloride Estimation—This test is indicated in any patient with severe vomiting, prolonged diarrhea, Addison's disease, muscle cramps, or profuse perspiration. It is of medicolegal value in determining whether death was due to drowning to do chloride estimations on blood from the right and left ventricles of the heart

1 **Normals**—These are 350 to 550 mg of sodium chloride per 100 cc of whole blood or 570 to 620 mg per 100 cc of plasma

2 **Interpretation**—There are no important causes of a high blood chloride. The causes of a low blood chloride are as follows

(a) *Loss of Hydrochloric Acid*—The commonest cause is vomiting, especially that of intestinal obstruction or pyloric stenosis. Gastric or duodenal fistula are less common causes. In some cases of acute or chronic dilatation of the stomach, a sufficient amount of gastric juice is retained in the stomach to lower the blood chlorides. Low blood chlorides frequently occur in patients being treated by continuous aspiration of the stomach. An alkali reserve is always indicated in addition to the blood chloride estimation in this group

(b) *Loss of Neutral Chlorides*—All causes of prolonged, profuse diarrhea or perspiration may produce this result. Severe muscle cramps¹ characterize the clinical picture. The syndrome of heat exhaustion may occur

(c) *Loss of Chlorides and Alkali*—Pancreatic fistula is the only cause. Acidosis is associated

(d) *Deficient Chloride Intake*—A restricted chloride intake may produce symptoms of chloride deficiency and frequently contributes to the lowered blood chloride in the above conditions. Sodium chloride is often restricted in the diet without sound reason. Edema is the only condition in which the evidence for benefit from restriction of sodium chloride seems adequate

(e) *Addison's Disease*—Low serum sodium and high potassium seem to be one of the most important disturbances in Addison's disease. Since the sodium level in the plasma parallels the chloride level, a low blood chloride is the rule in untreated Addison's disease. The level of the blood chloride may be used as a guide to therapy with sodium salts, low potassium diet and cortin

In groups (a) to (e) administration of sodium chloride is indicated

(f) *Loss of Chlorides into Exudates or Transudates*—This accounts for the lowered blood chloride in pneumonia and burns. Symptoms of

¹ Talbott J H Heat Cramps Medicine 14 323-376 (Sept) 1935

chloride deficiency rarely occur in pneumonia but are not uncommon after severe burns. Administration of chlorides is not indicated in pneumonia but large amounts of dilute sodium chloride solution by mouth or intravenously are of great value in the therapy of severe burns.

Chloride excretion in the urine is decreased in all the above conditions. A faint cloud in the qualitative test for chlorides in the urine is sometimes used as a point in favor of a diagnosis of lobar pneumonia.

(g) *Medicolegal Application*—A comparison of blood chloride estimations made on blood aspirated from the left and right ventricles of the heart will aid in a medicolegal decision as to whether death was due to drowning or the body was thrown into the water after death occurred. In drowning in fresh water, the blood of the left ventricle will have a markedly lower chloride content than the blood of the right ventricle, and in drowning in salt water, the opposite is true.

II DUODENUM AND PANCREAS

Duodenal contents consist of a mixture of the secretions of the duodenal mucosa, pancreatic juice, stomach contents, bile, and any transudates, exudates, or lower intestinal contents which may be added under pathologic conditions. Hence, the results are difficult of interpretation. Duodenal contents may be removed by the duodenal tube and examined for pancreatic ferments, or magnesium sulphate may be instilled according to the Meltzer-Lyon technic and the bile thus caused to flow may be withdrawn and examined. When the entrance of the tube into the duodenum is proved by fluoroscopy, failure to demonstrate more than a trace of bile pigment in the fluid withdrawn is good evidence for complete obstructive jaundice. The presence of cholesterol crystals or calcium bilirubinate granules in concentrated (B) bile is said¹ to suggest the presence of gall stones. Bacteriologic studies² of this bile have not proved reliable in the diagnosis of infections of the biliary tract.

The amylase³ concentration in the blood is increased in diseases of the pancreas which involve the acini or ducts. Amylopsin and other pancreatic enzymes may be absent from the duodenal contents in very extensive pancreatic disease or in obstruction to the pancreatic ducts.

III THE LIVER AND BILIARY TRACT

*A Resume of the Essential Points in Anatomy, Physiology, Biochemistry, and Pathology*⁴

¹ Rousselot, L. M. and Bauman, L. Cholesterol Crystals and Calcium Bilirubinate Granules: Their Significance in Bile Obtained Through the Duodenal Tube. *J. A. M. A.* 100: 254-256 (Jan. 28) 1933.

² Nauss, R. W., Lake, M. and Torrey, J. C. A Critical Analysis of the Lyon Bile Drainage Technic As An Aid to Bacteriologic Diagnosis. *J. Lab. and Clin. Med.* 17: 109-132 (Nov.) 1931.

³ Elman, R. Blood Amylase in Relation to Disease of the Pancreas. *Arch. Int. Med.* 48: 828-835 (Nov.) 1931.

⁴ Best, C. H., and Taylor, N. B. *The Physiological Basis of Medical Practice*. 1 p. 728-748. William Wood & Co. Baltimore, 1937.

Mann, F. C. The Effects of Complete and of Partial Removal of the Liver. *Medicine* 6: 419-511 (December) 1927.

Ivy, A. C. Physiology of Gall Bladder. *Physiol. Rev.* 14: 1-102 (Jan.) 1934.

in bile pigment in the blood may or may not occur, depending on the proportion of the biliary tract which is occluded. If it is completely occluded, no bile will enter the duodenum and jaundice will, of course, be present.

Partial occlusions anywhere in the biliary tract tend to produce dilatation above the point of obstruction. But a preceding inflammation which has resulted in scar tissue formation will prevent this dilatation. All types of obstruction in the biliary tract with the exception of obstructions in the cystic duct tend to produce increase in the bile pigments in the blood stream and to decrease the amount of bile entering the duodenum.

Complete obstruction in the common duct or in the ampulla of Vater is the commonest type of obstruction which occurs. It does not result in "hydrops" of the gallbladder but does produce jaundice.

Even in complete obstruction small amounts of bile pigment reach the intestinal tract indirectly by way of the blood stream and the various secretions which are poured into the digestive canal.

3 The Bile Pigments.—Bilirubin is formed from the destruction of hemoglobin wherever this occurs, but normally chiefly in the spleen, bone marrow, or liver. It is, however, in a form which will not pass through the kidneys into the urine and, theoretically at least, will not give the direct van den Bergh test. It is carried by the blood stream to the liver where it is transformed to a form of bilirubin which is excretable by the kidney and, according to the theory, will give the direct van den Bergh test. It is then normally excreted through the bile capillaries and ducts into the gallbladder, where the bile is concentrated to approximately one-tenth volume before it passes into the intestine. In the intestine the bilirubin is reduced by the action of colon bacilli to urobilinogen, formerly called hydrobilirubin. Most of this is excreted in the feces, giving to them their normal color, but some is reabsorbed and carried by the portal vein to the liver where it is changed back into bilirubin and reexcreted into the intestine. Even the slightest liver damage, however, permits that portion of the urobilinogen which reaches the damaged region in the liver to pass through into the systemic circulation, and this is excreted in the urine if renal function is not greatly impaired. After standing or on exposure to light the urobilinogen is gradually oxidized to urobilin. Bilirubin is reddish yellow in color but is readily oxidized to the green pigment biliverdin.

From the above facts the following generalizations are possible: (a) increased destruction of hemoglobin within the body will increase the bilirubin content both of the circulating blood and of the bile entering the intestine, the direct van den Bergh test should be negative and bilirubin will be absent from the urine. Urobilinogen in both stool and urine will be increased. This is the syndrome of hemogenous jaundice.

(b) Partial obstruction, whether in the bile tracts or the liver, will increase the bilirubin content of the systemic blood and decrease the bilirubin content of the bile entering the intestine. The direct van den Bergh test should be positive. The tests for bile pigments and urobilinogen in the urine will be positive and urobilinogen will be decreased in the stool.

¹ Editorial: Interrelation of Bile Pigment and Hemoglobin. J. A. M. A. 96: 1310 (18) 1931.

Rich. A. R. The Pathogenesis of the Forms of Jaundice. Bull. Johns Hopkins 147: 338-377 (Dec.) 1930.

(c) Complete obstruction will produce a greatly increased bilirubin content of the blood with a strongly positive direct van den Bergh test, and abundant bilirubinuria, but as no bile pigment enters the intestine no urobilinogen is formed, and, therefore, urobilinogen is absent from both stool and urine. The absence of urobilinogen from the urine in a case of obvious jaundice is the most conclusive test of a complete biliary obstruction. If urobilinogen is present, however, the existence of colon bacillus infection of the biliary or urinary tract must be ruled out before it can be concluded that complete obstruction is not present.

(d) Rapid passage of bile through the intestinal tract will not allow time for conversion of bilirubin to urobilinogen and some of it will be present in the stool as bilirubin or its oxidation product biliverdin.

B Function Tests¹—Tests designed to determine the ability of the liver to perform each one of its numerous functions have been devised,² but have not yet been adopted generally. Hypoglycemia³ is being found in some of the more severe types of liver damage. The tests interpreted below are those which give the most information but even these tests often fail to add anything to the information obtained from a good history and physical examination.

1 Bile Pigment (Bilirubin or Biliverdin) and Bile Salts in the Urine—These should always be tested for if clinical jaundice is present, if a cause of jaundice is suspected, or if the urine is dark yellow or brown in appearance. Bile pigments are much more frequently present than are bile salts so that pigment tests as well as bile salt tests should be done.

Positive tests for either pigments or salts or both, may be found in all the conditions listed below that show an icterus index above 15, except the hemogenous jaundice group. It must be remembered however, that hemolytic icterus (acholuric jaundice) strongly predisposes to the formation of gallstones and that gallstones may produce obstructive jaundice. Hence, bile pigment in the urine does not exclude the diagnosis of hemolytic icterus but its appearance after this diagnosis has been established should suggest the possibility of co-existent gallstones.

2 Urobilinogen⁴ in the Urine—This is much the most sensitive test of liver function available. It is so simple that it could with

¹Piersol G M and Rothman, M M. Practical Value of Liver Function Tests. A Comparative Study. J A M A, 91: 1768-1774 (Dec 8) 1928.

²O'Leary P A, Greene C H and Rowntree L G. Diseases of the Liver VIII. The Various Types of Syphilis of the Liver with Reference to Tests for Hepatic Function. Arch Int Med 44: 155-193 (Aug) 1929. The preceding articles in this series by Greene Rowntree and others are equally worthy of study.

³See references on page 144.

⁴Nadler W H and Wolfer J A. Hepatogenic Hypoglycemia Associated with Primary Liver Cell Carcinoma. Arch Int Med 44: 700-710 (Nov) 1929.

⁵Wallace G B and Diamond J S. The Significance of Urobilinogen in the Urine as

serum or plasma due to pigments derived from yellow and green vegetables. It differs from true jaundice in that the sclerae are not altered in color and the calluses of the palms and soles are most markedly yellow. It is especially common in diabetes mellitus because of the type of diet usually prescribed. The yellow pigment can be extracted from the blood serum or plasma with petroleum ether in which bilirubin is not soluble.

All interpretations given below presuppose that the increase is due to bilirubin.

(a) *Normals*—The normal values are 4 to 6 in either adults or children.

(b) *The Conditions Which May Produce an Icterus Index of 6 or 15* (clinical jaundice)—(1) Obstructive jaundice. This may result from any obstruction in the biliary tract preventing or impeding the outflow of bile. This is by far the commonest cause of a greatly increased icterus index. It is most often due to a stone in the common duct or to carcinoma of the head of the pancreas, but inflammation in the biliary tract, scar tissue contraction, metastatic malignancy in the adjacent lymph nodes, and numerous other conditions may produce this syndrome. A normal icterus index does not exclude any of these diagnoses although it does exclude any great degree of interference with the escape of bile from the biliary tract. This test should be frequently repeated in this group of cases to determine whether the tendency is to clear up as in stone or to persist as in carcinoma. Even icterus due to carcinoma may temporarily disappear, however, due to reabsorption of the perimalignant edema, or to sloughing of the portion of tumor tissue which is obstructing the duct.

(2) Hepatogenous jaundice. Diffuse liver damage is the usual cause. The degree of increase in the icterus index is very variable in this group.

An icterus index above 30 is the rule in "catarrhal jaundice" (really a diffuse hepatitis), in acute syphilitic hepatitis, in acute yellow atrophy of the liver (a very rare condition), in obstructive biliary cirrhosis (due to a chronic obstruction in the biliary tract), and in Hanot's cirrhosis (very rare).

An icterus index of 10 to 20 is the rule in Laennec's (also called portal, atrophic, or alcoholic cirrhosis) cirrhosis, Banti's disease, the various chronic forms of syphilis of the liver and in the liver damage occurring in pernicious vomiting, eclampsia, or in poisoning from chloroform, cinchophen, organic arsenicals, or phosphorus. In any of this group the icterus index may be normal or may rise to above 30. Such a high index in this group indicates a more serious prognosis.

Patchy lesions in the liver may produce any variation from no alteration in the icterus index (which is most common) to the most extreme increase, depending in part upon the amount of liver tissue destroyed, but chiefly on whether or not they happen to obstruct important bile passages. The important lesions in this group are primary and secondary malignant tumors, abscesses (pylephlebitic, cholangitic, pyemic, or amebic), gummata, tubercles, and echinococcus cysts of the liver.

(3) Hematogenous jaundice. This results from increased hemoglobin destruction within the body. Bile pigment is usually absent from the urine in this group. Icterus neonatorum,¹ erythroleukoblastosis, pulmonary infarction, and familial hemolytic icterus are the only conditions in which clinical jaundice due to excessive blood destruction is the rule. It may occur, however, in any of the causes listed below for latent hematogenous jaundice, and in phenylhydrazine poisoning, due in part to blood destruction and in part to toxic action on the liver.

(c) *The Conditions Which May Give Rise to an Icterus Index of 6 to 15 (latent jaundice not clinically detectable)*—The chief value of the test is its ability to detect these slight grades of increase in the bile pigment in the blood.

(1) Partial or early obstruction in the biliary tract. The causes are the same as those listed under (b) (1), but latent icterus is found only in the stages of onset or of disappearance of the obstruction, or if the obstruction remains slight.

(2) Disease of the liver. The great majority of the cases belonging in this group (see (b) (2) above) are characterized by latent icterus.

(3) Blood destruction. Latent icterus is the rule in this group with the exceptions noted under (b) (3) above. The other important causes are the following: pernicious anemia (differentiates it from non-hemolytic anemias in which the icterus index is low), other hemolytic anemias, malaria, the reabsorption of internal hemorrhages (ruptured ectopic pregnancy, postoperative hemorrhages, etc. may often be suspected if an otherwise unexplained rise in the icterus index occurs), and reabsorption of pigment from hemorrhagic infarcts or from passively congested lungs (brown induration). The last condition not infrequently gives rise to a high icterus index in heart disease. Clinical jaundice in heart disease (icterus index above 15) usually means extensive pulmonary infarction, although the passive congestion of the

¹ Snelling C. E. Icterus Neonatorum. J. Ped. 2: 399 (April) 1933.

Gordon M. B., and Kemelhor M. C. Icterus Neonatorum. J. Ped. 2: 685 (June) 1933.

lungs and liver is undoubtedly also of importance. An increase in the icterus index favors hemorrhage, rather than thrombosis, embolism or spasm, as the pathology underlying a cerebral vascular accident. An increase in the icterus index favors dissecting aneurysm rather than coronary occlusion as the cause of sudden severe pain in hypertensive disease.

The increase in the icterus index in pneumonia, typhoid fever, and other infectious diseases has the same serious import long given to jaundice in these diseases. It is claimed that in known peptic ulcer a high index suggests a site in the duodenum rather than in the stomach. The roentgen ray examination is much more reliable for this localization.

(d) *The Causes of an Index Below Normal*—These include all cases in which there is a lessened blood destruction within the body, hence, all types of anemia due to loss of blood from the body or to deficient function of the bone marrow.

Undoubtedly in many cases there are various combinations of biliary obstruction, liver damage, and blood destruction, all playing a part in the production of the increased bile pigment level in the blood.

4 *The van den Bergh Test¹ for Bilirubin in Plasma or Serum*—This is supposed to distinguish between bilirubin that has passed through the liver (obstructive jaundice) which gives the direct reaction and bilirubin that has not passed through it (hemolytic jaundice) which gives only the indirect reaction. A quantitative estimation of bilirubin is possible but is more difficult and less accurate than the icterus index determination.

The icterus index is recommended as the quantitative test with a qualitative direct van den Bergh to be done in addition on those rare cases in which the clinical findings do not indicate whether the icterus is hemolytic or obstructive in type, remembering that a negative direct reaction is common even in the cases of partial obstruction when the icterus index is still low.

(a) *Interpretation*—Normals should give only an indirect reaction and an estimation of 0.05 mg. to 0.25 mg. per 100 cc. Actually, normals usually give a color too pale to read at all. A direct reaction supposedly indicates obstructive jaundice and is usually associated with a high indirect reaction and an increased icterus index. A biphasic or delayed direct reaction is believed by some to indicate disease of the liver itself. Absence of the direct reaction is supposed to rule out obstructive jaundice. An increased indirect reaction is supposed to occur in all types of hemolytic icterus whether latent or obvious (pernicious anemia, acholuric jaundice, etc.). In my experience the clinical study of the patient is more apt to give correct results than is the van den Bergh test. My clinical results with this test agree with the experimental observations of Snider and Reinhold² in suggesting that the character of the van den Bergh reaction (direct, delayed, biphasic indirect) depends

¹ Barron E. J. G. and Brumstead J. H. The Pathogenesis of Early Obstructive Jaundice. *J. of Exper. Med.* 47: 999-1012 (June) 1928.

² Snider H. F. and Reinhold J. G. A New Interpretation of the Van den Bergh Reaction. *Am. J. Med. Sc.* 180: 248-255 (Aug.) 1930.

on the concentration of bilirubin in the blood stream rather than on the mode of production of the increase and that, consequently, it adds little if any information to that derived from the icterus index

5 **Dye Excretion Tests**—Many such tests have been proposed, but the bromsulphalein test¹ seems the most satisfactory. It is indicated when severe diffuse liver damage is suspected for any reason. The results will usually confirm a previously formed clinical impression rather than give any really new information. Dye retention parallels jaundice so closely that little additional information is derived by doing it in patients showing clinical icterus.

(a) *Normals*—Complete disappearance of the dye from the blood occurs within 30 minutes. Since patchy liver damage may give normal results a pathologic result is more significant than a normal one.

(b) *Interpretation*—More than a trace of dye at 30 minutes is abnormal. The per cent of dye retention indicates roughly the amount of liver damage.

The causes of impaired dye excretion aside from obstruction to the biliary tracts are acute yellow atrophy of the liver, cinchophen phosphorus, chloroform or arsphenamin poisoning, eclampsia, cholecystitis and cholangitis, cirrhosis of all types, carcinoma of the liver, Weil's disease, yellow fever, and others. The degree of impairment depends more on the proportion of total liver tissue involved than on the severity of the lesion, e.g., "catarrhal jaundice" (actually a diffuse hepatitis) shows greater impairment of function as a rule than does primary or secondary carcinoma of the liver because the normal liver tissue intervening between the cancer nodules is able to carry on the liver function quite well.

Graham² has suggested the use of Phenol tetraiod phthalein Sodium (Iso Iodeikon) as a combined dye test of liver function and radio-opaque substance for use in roentgenologic study of the gall bladder by the Graham Cole technic. The test is convenient whenever both studies are desired. The directions accompany the dye.³ Normal values are retention of 10 per cent or less at 30 minutes and 5 per cent or less at one hour and the interpretation is the same as that given for the bromsulphalein test.

6 **Galactose Tolerance Test**—This test was introduced by Richard Bauer as a method for distinguishing diffuse liver damage such as occurs in hepatitis or cirrhosis from patchy lesions in the liver or obstructive jaundice. It may be done in all cases in which such problems arise. The normal result is an excretion of less than 3 gm. of galactose in the urine after the oral administration of 40 gm. Unfortunately, the original hope that it would distinguish between obstructive and

¹ Cantarow A. The van den Bergh Reaction and the Bromsulphalein Test in the Estimation of Hepatic Functional Impairment. *Am J Med Sci* 184: 228-240 (August) 1932.

² Graham I. A. Cole W. H. Copher G. H. and Moore S. Diseases of the Gall bladder and Bile Ducts. 1p. 337-308. Lea and Febiger Philadelphia 1928.

³ Obtainable from Mallinckrodt Chemical Works, St. Louis Mo.

⁴ Kosenberg D. H. The Galactose and Urobilinogen Tests in the Differential Diagnosis of Obstructive and Intrahepatic Jaundice. *Ann Int Med* 8: 60-71 (July) 1934.

Tumen H. J. and Piersol C. M. The Value of Alimentary Calactosuria in the Diagnosis of Jaundice. *Ann Int Med* 7: 311-329 (Sept.) 1933.

Banks B. M. Sprague P. H. and Snell A. M. Clinical Evaluation of the Galactose Tolerance Test. *J A M A* 100: 108-1093 (June 4) 1933.

Shay H. and Lieben I. The Galactose Tolerance Test in Jaundice: a Consideration of the Evidence Permitting the Measurement of Galactose Utilization by Urinary Excretion. Some Sources for Error in its Interpretation and an Addition in Routine Technic. *Ann Int Med* 10: 129-1303 (Mar) 1937.

intrahepatic jaundice has not been borne out, possibly because common duct stones are so frequently associated with some degree of hepatitis. The greater the excretion of galactose in the urine the more probable is it that there exists diffuse hepatic damage. An excretion of over 5 gm of galactose rarely occurs unless hepatitis or diffuse liver damage is present. Such a positive galactose test is probably the most conclusive laboratory sign of diffuse injury to the hepatic cells. However, a normal test does not exclude this nor does a high result prove that the jaundice is not of the obstructive type.

7 Tests Discussed in More Detail in Other Chapters Which Are of Value in Liver Disease —(a) *Tyrosin¹ and Leucin in the Urine* —These may be detected as crystals on microscopic examination, or tyrosin may be suspected if a positive Milon's test is secured on protein free urine. Their presence indicates severe diffuse liver disease such as occurs typically in acute yellow atrophy, but small amounts have been found in 'catarrhal jaundice'.

(b) *Ascitic Fluid* —This appears in passive congestion of the liver, Laennec's cirrhosis, syphilitic cirrhosis and late in the course of Banti's disease, but is uncommon in other types of liver disease. It should be studied and will be found to have the characteristics of a transudate in cases of these types which are uncomplicated by peritonitis or malignant tumors. Sudden increase in the amount of fluid in a case of Laennec's cirrhosis, particularly if the fluid also changes in character, should suggest the possibility of a primary carcinoma of the liver superimposed on the cirrhosis. Rapid reaccumulation of ascitic fluid over a long period of time approaching an exudate in character, should suggest the rare extensive perihepatitis and perisplenitis, usually called "Zuckerguss" liver and spleen.

(c) *The Congo Red Test* —This should be done if amyloidosis is considered to be a possible cause of the liver involvement.

(d) *Increase in the Ammonia Nitrogen at the Expense of the Urea Nitrogen in the Urine* —This occurs in the more severe forms of liver disease as well as in some types of acidosis and cystitis.

8 Other Liver Function Tests ²—A great many liver function tests have been developed and more constantly appear. Most of these are either too expensive or troublesome to be practical or have not demonstrated any advantages over the tests given above or are so new that an accurate appraisal is not yet possible.

¹Lichtman S S. Origin and Significance of Tyrosinuria in Disease of the Liver. Arch Int Med 53 680-688 (May) 1934.

²Yegge W B. A Critical Review and Evaluation of Tests for Liver Function. Ann Int Med 8 907-919 (Feb) 1935.

Cantarow, A., and Nelson J. Serum Phosphatase in Jaundice. Arch Int Med 59 1045-1050 (June) 1937.

Flood C A, Gutman Ethel B. and Gutman A B. Phosphatase Activity Inorganic Phosphorus and Calcium of Serum in Disease of Liver and Biliary Tract. A Study of One Hundred and Twenty three Cases. Arch Int Med 59 981-999 (June) 1937.

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Soffer L J., and Paulson M. Comparative Advantages and Further Modification of the Bilirubin Excretion Test for Hepatic Function. Am J M Sc 192 535-540 (Oct) 1936.

Soffer L J., Dantes D A. and Sobotka H. Utilization of Intravenously Injected

IV THE INTESTINAL TRACT

A Resume of Essential Points in the Physiology,¹ Biochemistry, and Pathology

The chief function of the small bowel is the absorption of the end products of digestion. The end products of carbohydrate digestion are monosaccharides, of protein digestion, amino acids, and of fat digestion, fat acids and glycerol. Much of this digestion takes place during the time of passage through the upper portion of the small bowel. In addition, bile salts, most drugs, part of the water, and some urobilinogen are absorbed. The time for the passage of intestinal contents from the stomach into the large intestine is normally 3 to 6 hours. Passage through the large intestine requires much more time, usually 24 to 30 hours. The total time from ingestion of a meal to defecation of the residue therefrom (best detected by ingestion of 0.3 gm. of carmine in capsules) is, therefore, normally 30 to 40 hours, but only periods under 24 hours or over 48 hours should be regarded as deviations from the normal.

The composition of the intestinal contents is extremely complex. It varies progressively during the passage through the intestinal tract. The contents of the duodenum have been mentioned. As this complex fluid mixture passes through the jejunum and ileum, digestion and absorption progress as above outlined until relatively little nutritive material remains when the mixture reaches the cecum. The number of bacteria progressively increases as the ileum is traversed and suddenly mounts to about 50 per cent of the solid content in the large bowel. The contents of the cecum and transverse colon are still fluid, but water is absorbed at such a rate in the large bowel that the contents of the splenic flexure and sigmoid are normally semifluid or formed and the normal stool as passed is sufficiently soft to be readily molded during its expression through the anal sphincter and yet firm enough to retain its form. About 50 per cent of the dry weight of the stool consists of bacteria (mostly dead). The remainder con-

Sodium *d* Lactate as a Test of Hepatic Function. Arch Int Med 62 918-924 (Dec) 1938

Soffer L J, Dantes D A, Newburger R and Sobotka H. Metabolism of Sodium *d* Lactate. II. Utilization of Intravenously Injected Sodium *d* Lactate by Patients with Acute Diffuse Parenchymal Injury of the Liver. Arch Int Med 60 882-886 (Nov) 1937

Boyce F F, and McFetridge Elizabeth M. Studies of Hepatic Function by the Quick Hippuric Acid Test. I. Biliary and Hepatic Disease. Arch Surg 37 401-426 (Sept) 1938

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Althausen T L, Gunther L, Lagen J B and Kerr W J. Modification of the Dextrose Tolerance Test as an Index of Metabolic Activity of the Liver. Arch Int Med 46 482-493 (Sept) 1930

Ricketts H T. Function of the Liver. An Appraisal of the Modified Dextrose Tolerance Test. Arch Int Med 52 147-157 (July) 1933

Rowe A W, Plummer A J and McManus Mary A. The Metabolism of Levulose. I. Some General Considerations on Provocative Levulosuria. Am J Med Sci 180 15 (July) 1933

Stowe W P, Delprat G D and Weeks A. The Rose Bengal Test of Liver Function. Am J Clin Path 3 55-60 (Jan) 1933

¹ Hewlett A W. Pathological Physiology of Internal Diseases. Pp 181-213. Ed 3. D Appleton and Company New York 1928

bacillary dysentery, or any purulent inflammation in, or communicating with, the large intestine. Starch, fat, fat acids and soaps, and muscle fibres may be present in increased amounts in any form of diarrhea in which the small intestine participates in the increased peristalsis, or when abnormal communications exist between the stomach or jejunum and the colon, or when serious disease of the pancreas or obstruction to its ducts prevents adequate amounts of pancreatic juice from reaching the intestine. The presence of many undigested nuclei after ingestion of a liver meal is suggestive of a deficiency of pancreatic secretion. Usually the clinical diagnosis of pancreatic disease is made before it becomes sufficiently extensive to produce these changes. Recent ingestion of mineral oil must be excluded as it gives the usual tests for fat. Excessive amounts of fat, fat acids and soaps are, also, the rule in complete or nearly complete obstruction in the biliary tract.

The absence of cornified epithelium in meconium is diagnostic of congenital atresia¹ in the alimentary tract, since normally such cells are present due to swallowing of amniotic fluid containing desquamated epithelium from the fetal skin.

A search for ova should be included in the microscopic study in all stool examinations.

E Intestinal Parasites and Their Cysts or Ova²⁻¹ **Indications for This Examination**—Since routine examination of the feces for intestinal parasites and their cysts or ova is rewarded in an appreciable percentage of cases by finding them (even in temperate climates), such an examination is now the rule in some of the best hospitals and clinics.

Eosinophilia, unexplained anemia and gastrointestinal symptoms are the chief general indications for this examination. A few of the more specific indications follow.

Chronic or recurrent diarrhea, especially if associated with the passage of blood and mucus, is the chief indication for a search for amebae, *Balantidium coli*, ova of *Fasciolopsis buski*, or flagellates. The possibility of amebic ulceration in the colon should not be overlooked in searching for the focus of infection in such diseases as chronic infectious arthritis.

¹ Farber S. Congenital Atresia of the Alimentary Tract. Diagnosis by Microscopic Examination of Meconium. J A M A 100 1753-1754 (June 3) 1933.

² A complete discussion of parasitology would require a book length treatise. Hence anyone who wishes to go more deeply into this subject should consult one of the excellent works on parasitology and tropical medicine now available. Among the best is Craig C F and Faust E C. Clinical Parasitology. Pp 733. Lea and Febiger Philadelphia 1937. Space permits only a brief consideration of some of the more important parasites of man.

Vague nervous symptoms in children or the observation of "worms" in the stool are the chief additional indications for examination for parasitic worms or their ova

Pruritus ani, especially in children, should cause one to search for *Oxyuris vermicularis* and its ova

The presence of a chronic foamy leukorrhea should cause one to examine the vaginal secretion for the flagellate *Trichomonas vaginalis*

Fever with muscle pains, edema of the eyelids, and eosinophilia indicates a search for *Trichinella spiralis* in the feces, blood, spinal fluid, and muscles

A cystic tumor in the internal organs of a sheepherder or other person intimately in contact with dogs should arouse the suspicion of echinococcus cyst

Hematuria in a patient who has been in Egypt recently should lead one to search for ova of the *Schistosoma hematobium* in the urine. Some cases of bilharziasis have occurred in the southern states

2 Precautions to Be Observed in Collection of Specimens —

Do not forget that if active amebae or flagellates are to be looked for with any great chance of success, the stool must be fluid or semifluid and reach the laboratory as soon as possible after it is passed and while still warm. Hence, specific orders relative to these points must be given. The second stool after a saline cathartic is most suitable for flagellates and is satisfactory for amebae. Amebae will be most frequently found in mucus or secretions removed with the aid of the proctoscope or sigmoidoscope from the base of the ulcers. *Oxyuris vermicularis* ova are most easily found in scrapings from the perianal folds or from under the finger nails

3 Interpretation — Since some intestinal parasites are probably not pathogenic and even the most pathogenic may be present without producing symptoms, the demonstration of the presence of a parasite does not prove that that parasite is responsible for the condition for which the patient is seeking relief

(a) *Rhizopoda* or *Amebae* ¹—*Endameba histolytica* may account for an acute or chronic dysentery, for vague gastrointestinal symptoms or more rarely for abscesses in the liver, lungs, brain, or other organs. The *Endameba coli*, *Iodameba williamsi*, and *Endolimax nana* are non

¹ Kessel J. F. and Mason V. R. Protozoan Infection of the Human Bowel. A Comparison of Laboratory and Clinical Observations. J. A. M. A. 94, 1-6 (Jan 4) 1930

James W. M. Human Amoebiasis Due to Infection with *Entamoeba histolytica*. Ann. Trop. Med. and Parasitology 22, 201-258 (Aug 28) 1928

Special Articles on Amebic Dysentery. J. A. M. A. 102, 1636-1641 (Nov 18) 1933
Amoebiasis Outbreak in Chicago. Report of a Special Committee. J. A. M. A. 102, 369-372 (Feb 3) 1934

pathogenic but so commonly present that it is necessary to be able to differentiate them from *Endameba histolytica*. If large numbers of active amebae are present together with mucus and blood, the diagnosis of amebic dysentery is readily established. In the less active stages the organisms will be fewer and in the quiescent stages chiefly encysted and the diagnosis is less simple. If only cysts are present with no symptoms, the identification is difficult but important, as these carriers constitute a public health menace. If any doubt exists as to the correct identification it should be made by an expert in parasitology. The complement fixation test¹ is of value in research studies but a positive test does not prove that symptoms are due to amebiasis.

(b) *Flagellates*—*Giardia lamblia*, *Chilomastix mesnili*, and *Trichomonas hominis* have been found in the feces of from 3 to 20 per cent of persons living in temperate climates and they are still more frequent in the tropics. They have each been claimed to produce a mild chronic intestinal irritation with diarrhea as the chief symptom and they have each been claimed to be non pathogenic. Etiologic importance should certainly not be attributed to them unless large numbers of the active form are present and no other cause for the symptoms has been determined after a thorough study. The *Trichomonas vaginalis* is frequently present in the vagina and it is possible that it is responsible for some cases of chronic vaginitis² with leukorrhea.

(c) *Ciliates*—*Balanitidium coli* is the only ciliate affecting man. It is an occasional cause of diarrhea in farmers. It is commonly found in the feces of hogs but apparently causes no symptoms.

(d) *Cestodes, Flat, or Tape Worms*³—The *Taenia saginata* (beef tape worm), *Taenia solium* (pork tape worm), *Hymenolepis nana* (dwarf tape worm), and *Diphyllobothrium latum* (fish tape worm) are the important members of this group in which the adult form occurs in man. The *Taenia solium* is rare in the United States, but all the others are frequently found. Loss of weight and vague nervous or gastro intestinal symptoms are said to result from heavy infestations. Normocytic anemias of varying severity with or without an eosinophilia may be produced by these worms, particularly the *Diphyllobothrium latum*. Usually, however, there are no detectable changes aside from finding of the segments or ova in the stool. The *Diphyllobothrium*

¹ Paulson M., and Andrews J. Complement Fixation in Amebiasis. A Comparative Evaluation in Clinical Practice. Arch Int Med 61: 562-578 (Apr.) 1938.

Craig C. F. Observations Upon the Practical Value of the Complement fixation Test in the Diagnosis of Amebiasis. Am J Pub Health 27: 689-693 (July) 1937.

² Mathieu A. Trichomonas Vaginalis. A Cause of Vaginitis. Northwest Med. 29: 15-18 (Jan.) 1930.

³ Brown H. W. Intestinal Parasitic Worms in the United States. Their Diagnosis and Treatment. J A M A 103: 651-660 (Sept. 17) 1934.

Sunkes E. J. and Sellers T. F. Tapeworm Infestations in the Southern United States. Am J Pub Health 27: 893-898 (Sept.) 1937.

latum may cause a very severe anemia¹ which is difficult to distinguish from pernicious anemia, but it has not been sufficiently stressed that only a small percentage of persons infested with this worm develop any anemia and only about 0.1 per cent of infested persons develop a macrocytic anemia closely resembling pernicious anemia

Diphyllbothrium latum infestation was formerly uncommon outside of Finland, but the frequency in the United States is increasing, particularly in the lake regions of the north central states

The *Taenia echinococcus* (dog tapeworm) may pass its larval stage in man, producing cysts in the liver, lungs, brain, or other internal organs. The diagnosis of this rare condition is made by the complement fixation test. Puncture is dangerous as it may lead to anaphylactic shock or dissemination. The presence of typical hooklets in the cyst fluid removed at operation confirms the diagnosis

(e) *Nematodes or Round Worms* —

(1) *Ascaris lumbricoides* or large round worm. Infestation with this worm is common. It may explain nervous or gastrointestinal symptoms in children but usually produces no symptoms. These worms have a curious tendency to wander into the bile ducts or appendix and so may cause jaundice or appendicitis in rare instances. Occasionally such enormous numbers are present as to cause intestinal obstruction

(2) *Enterobius (Oxyuris) vermicularis*, also called the pin worm, seat worm or thread worm. These occur in the rectum and colon, especially in children, causing symptoms of pruritus ani

(3) *Necator americanus* or American hookworm. Infestation with these worms is common in the southern United States. It causes a severe hypochromic microcytic anemia and general maldevelopment

(4) *Ankylostoma duodenale*, the European or Old-World hook worm. Infestation with hookworm is an extremely common cause of severe hypochromic microcytic anemia and general undernutrition and maldevelopment in tropical and subtropical countries

(5) *Trichinella spiralis*². An acute gastroenteritis followed by fever, edema of the eyelids, muscular pains and eosinophilia coming on after eating improperly cooked pork may be explained by the finding

¹ Birkeland I W. Bothriocephalus Anemia. *Diphyllbothrium Latum* and Pernicious Anemia. *Medicine* 11: 1-139 (Feb) 1932

Magath T B. The Relation of *Diphyllbothrium Latum* Infestation to Public Health. *J A M A* 101: 337-341 (July 29) 1933

² Hall A A. Outbreak of Trichinosis in Central Ohio and the Use of the Bachman Intradermal Skin Test. *Ann Int Med* 10: 1544-1550 (Apr) 1937

Hall M C. Studies on Trichinosis VI. Epidemiological Aspects of Trichinosis in the United States as Indicated by an Examination of 1 000 Diaphragms for Trichinae. *Pub Health Rep* 53: 1086-1105 (July 1) 1938

of larvae of this parasite in the muscles, or rarely in the blood or spinal fluid. Larvae may often be recovered in the feces if a saline cathartic is given during the stage of acute enteritis, although this fact is not mentioned in most books. Infestations too mild to produce clinical symptoms are probably very frequent. Unexplained eosinophilia should lead one to consider this diagnosis. The Bachman intradermal skin test is of value.

(6) *Trichuris trichiura*, *Trichocephalus dispar*, or whip worm. They usually produce no symptoms, but have been thought to cause anemia or enteritis in rare instances.

(7) *Strongyloides stercoralis*. Discovery of the larvae in the stools may explain a chronic diarrhea.

(f) *Trematodes or Fluke Worms* —

(1) *Schistosoma hematobium* (*Bilharzia hematobia*). This organism lives in the veins of the bladder producing inflammation and hematuria. The ova are discharged in the urine. Bilharziasis is very common in northern Africa but rare elsewhere. A few cases have occurred in the southern states.

Schistosoma mansoni inhabits the veins of the portal system and the ova are found in the feces.

(2) *Fasciolopsis buski*. This is common in India, China, and Japan. It inhabits the duodenum producing a bloody diarrhea.

(3) *Clonorchis sinensis*. This is a common parasite of cats and dogs and of man in China and Japan.

(4) *Opisthorchis felinus*, the cat fluke. It occasionally infests man.

V SUMMARY OF THE DIFFERENTIAL DIAGNOSIS OF THE MORE IMPORTANT DISORDERS OF THE GASTROINTESTINAL TRACT

Very few of these diagnoses can be established by laboratory methods alone, but valuable evidence supplementing that obtained from the history, physical examination, roentgenologic study and gastroscopic study can be obtained in each instance. The accuracy of therapeutic control and of prognosis can be materially increased.

A Diseases — 1. **Carcinoma** — Hypochromic microcytic anemia of chronic blood loss with persistent blood in the stool is common in all ulcerated carcinomas and may be the first evidence detected. It is especially severe in carcinoma of the cecum and ascending colon. If no other symptoms or signs are present, careful study of the cardiac portion of the stomach by fluoroscopy after a barium meal with the head lower than the feet will often locate the carcinoma.

Normocytic anemia may occur late in the types of carcinoma not associated with bleeding (scirrhus carcinoma or carcinoma of the liver or pancreas) or anemia may be absent in such cases.

Achlorhydria or hypochlorhydria especially if associated with lactic acid formation, Boas Oppler bacilli or evidences of stasis (location near pylorus) strongly suggests carcinoma of the stomach but absence of these findings does not exclude this diagnosis

Impaired liver function, obstructive jaundice, or ascites of either exudate or transudate type may occur in primary or secondary carcinoma of the liver but the presence of any or all of these does not establish the diagnosis and their absence does not exclude this diagnosis

Persistent complete obstructive jaundice suggests carcinoma of the head of the pancreas or biliary tract but may be absent or, in rare instances, disappear after it has developed

2 **Peptic Ulcer**¹—Intermittent blood in the stools, normocytic anemia of acute hemorrhage or hypochromic microcytic anemia of chronic blood loss and hyperchlorhydria are most commonly found, but one or all of these may be absent. The localization in the duodenum, stomach, or esophagus must be made by other examinations. A large volume of stomach contents with sarcinae and food residues suggests obstruction at the pylorus and indicates study of the alkali reserve, blood chlorides, and urea or non protein nitrogen of the blood as guides to therapy

Alkali reserve estimations, stool examinations for blood, and gastric analyses for free hydrochloric acid and pepsin should be repeated during treatment with alkalis to make sure that the objects of therapy are being attained without the production of an alkalosis. Serum protein estimations may be desirable before operation to be sure that the level is adequate for proper wound healing²

3 **Intestinal Obstruction**—The alkali reserve, chloride, and urea nitrogen estimations on the blood are the most important guides to preoperative preparation but do not establish the diagnosis. Normal findings with these tests do not exclude this diagnosis. A stool consisting largely of blood passed after the diagnosis of intestinal obstruction has been established suggests intussusception as the cause. Erythrocytosis (increase in hemoglobin and red cells) indicates dehydration requiring intravenous or subcutaneous fluid administration. Normal or low hemoglobin or red cell count does not exclude anhydremia however

4 **Ulceration of the Small or Large Intestine**—Occult or gross blood in the stool which is often fluid or semifluid is the most constant finding. If outside the stool and if hemorrhoids or carcinoma of the

¹ Mann F. C. *Physiologic Mechanisms in Relation to the Development of Peptic Ulcer*. Minnesota Med 20 755-761 (Dec) 1937

² See references on page 41

rectum have been excluded, a location in the rectum or sigmoid, indicating a proctoscopic or sigmoidoscopic examination, is suggested. The type of anemia as determined by the color, volume, and saturation indexes will tell whether hemorrhage or infection is playing the greater role in its production.

Mucus without pus is suggestive of amebic dysentery. This diagnosis will be confirmed if active *E. histolytica* are found.

A Widal test, culture of the stool and blood for typhoid bacilli and of the stool for dysentery bacilli or for Barger's diplococcus,¹ and stains and guinea pig inoculation for tubercle bacilli (which may come from swallowed sputum) are further diagnostic aids worthy of consideration.

Pus in considerable quantities is more common in bacillary dysentery or ulcerative colitis.

The white and differential cell counts and the sedimentation rate will be of aid in this group. Simple leukopenia occurs in typhoid (no eosinophils) and in tuberculosis (eosinophils may be present) of the intestine. Eosinophilia is often associated with amebic colitis. Other types show normal counts or neutrophilic leukocytosis of varying degree. The degree of increase in sedimentation speed will correspond approximately to the extent of the ulceration in any type.

If sulfanilamide is being administered the level of the drug in the blood should be checked.

5 Cholecystitis and Cholelithiasis—Leukocytosis with rapid sedimentation rate characterizes the more acute inflammations. Stones in the common duct usually produce the syndrome of partial obstructive jaundice alternating with complete obstructive jaundice, but many stones may be present in the common duct without producing any icterus at all. Examination for gallstones by straining of all feces passed for several days after an attack of biliary colic will occasionally establish the diagnosis in doubtful cases, but failure to find stones does not exclude this diagnosis. Cholesterol or calcium bilirubinate crystals in Lyons drainage suggest the presence of stones. Absence of bile in the drainage fluid, if the position of the tip of the tube is correct, indicates complete obstruction during the period of the biliary drainage.

A severe normocytic anemia is not infrequently associated with chronic cholecystitis.

In jaundiced patients the prothrombin time should be brought to normal by administration of vitamin K and bile salts before operation.

6 Cirrhoses of the Liver—All types are comparatively uncommon, and some are extremely rare. Laennec's (also called atrophic,

¹ The relationship of this organism to ulcerative colitis is not yet established.

portal, or alcoholic) cirrhosis is by far the commonest form. An icterus index in the range of latent jaundice, less commonly high, with an increase in the urobilinogen in the urine are the only early laboratory findings. Later, ascites with characteristics of a transudate, impaired liver function as determined by the dye or galactose tolerance tests, and blood in the stool (hemorrhoids, esophageal varices) or hematemesis (esophageal varices) usually develop. Normocytic or macrocytic anemia and changes in the coagulation or prothrombin time may occur. Terminal marked increase in the icterus index and transformation of the characteristics of the ascitic fluid to those of an exudate (peritonitis, often tuberculous) are not uncommon. A sudden increase in the rate of accumulation of the ascitic fluid should cause one to consider the possibility of a primary carcinoma of the liver superimposed on the preexisting cirrhosis.

Banti's disease shows similar laboratory findings with the exception that normocytic anemia and simple leukopenia occur before changes in liver function are detectable, and that hematemesis more often dominates the picture.

Portal or splenic thrombosis is indistinguishable from Laennec's cirrhosis by laboratory tests, but impaired liver function is not usually present.

Syphilitic cirrhosis of the liver gives a similar laboratory picture to Laennec's cirrhosis, but with a slightly greater tendency to clinical jaundice with an icterus index above 15. Syphilis is unlikely to be the cause of a cirrhosis if the serologic tests for syphilis are negative. Positive tests suggest but do not prove a syphilitic etiology. Hepatitis from the organic arsenicals used in the treatment of syphilis is common.

Hypertrophic obstructive biliary cirrhosis shows the laboratory syndrome of partial or complete obstructive jaundice and also dye retention and increased galactose excretion indicating impaired liver function. Ascites may or may not be present.

Hanot's cirrhosis is extremely rare and is often confused with the preceding type. The laboratory picture is that of moderate to severe partial obstructive jaundice without ascites or blood in the stool.

Cardiac cirrhosis, or Pick's pericarditic cirrhosis gives rise to the laboratory findings described for Laennec's cirrhosis, but ascites occurs earlier and the ascitic fluid more nearly approaches an exudate in character.

A large number of cirrhotic changes occur in the liver which do not fit either clinically or pathologically into the usual classification of liver disease.

The clinical findings are necessary to complete the picture of any type of cirrhosis. The urine volume charted against the fluid intake will aid in controlling therapy designed to reduce ascites.

7 Hepatitis¹ and Diffuse Liver Damage from Toxins or Poisons—These cases also show varying degrees of the partial obstructive jaundice syndrome, including dye retention. The cases with the severest liver damage will show in addition a positive galactose tolerance test, increased amino acids in the urine with a positive Milon's test, and rarely leucin and tyrosin crystals, disturbances in the coagulation time of the blood, increase in the relative proportion of the total nitrogen of the urine in the form of ammonia nitrogen, and possibly hypoglycemia.

"Catarrhal jaundice" or acute hepatitis is the commonest condition in this group. Weil's disease, and yellow fever are rare febrile diseases with a similar picture. In any of this group the complete obstructive jaundice syndrome may develop.

Eclampsia and pernicious vomiting of pregnancy are discussed in Chapter XI. Changes in liver function may occur but are of little aid. Icterus is usually absent.

Syphilitic and postarsphenamine (other organic arsenicals may also produce it) hepatitis are common. Dye retention is more marked in proportion to the icterus than in other types of jaundice.

Less common causes of a similar syndrome are cinchophen, chloroform, phosphorus, carbon tetrachloride and phenylhydrazine poisoning.

Acute yellow atrophy or icterus gravis. This is probably simply a hyperacute destructive hepatitis which may result from any of the conditions here listed. It is very rare but relatively more common in pregnant women than in other individuals. The most extreme impairment of liver function occurs in this condition.

The differential diagnosis of these different types of hepatitis is largely clinical.

B Syndromes—**1 Complete Obstructive Jaundice**—This is characterized by clinical jaundice, clay colored stools in which the tests for urobilinogen and bile pigment are negative, dark urine containing bilirubin but no urobilinogen, and absence of bilirubin from fluid aspirated from the duodenum. The icterus index is usually over 50 and may be as high as 200. The most valuable evidence of complete obstruction is the negative urobilinogen in the urine with positive tests for bilirubin. This syndrome is present whenever bile is prevented

¹Weir J. I. Hepatitis. Some Forms Not Commonly Recognized. J. A. M. A. 111: 1356-1336 (Oct. 8) 1938.

from entering the intestinal tract no matter what the cause of the obstruction. The obstruction may be due to carcinoma of the head of the pancreas, the biliary tract or the adjacent lymph nodes, to stone, to scar tissue contraction, to enlargement of the nodes due to lymphadenitis, metastases, Hodgkin's disease, lymphosarcoma or leukemia, or to a sufficient degree of hepatitis or liver edema to obstruct the flow of bile within the liver. It may also occur from tumors of the liver so placed as to obstruct both hepatic bile ducts. In all cases, dye excretion is impaired but, if no diffuse liver damage is associated, the galactose tolerance test is normal.

2 Partial Obstructive Jaundice—This syndrome is characterized by an icterus index of 6 to 150 and often the presence of bilirubin in the urine. It differs from complete obstruction in the presence of urobilinogen in the urine and feces, and of bilirubin in the duodenal fluid. Dye excretion varies with the icterus index and there is no indication for this determination. The galactose tolerance test is normal if liver damage is not associated. It differs from hematogenous jaundice in that bilirubin may appear in the urine and a direct van den Bergh test may be obtained on the blood serum.

3 Hepatocellular Jaundice—This is characterized by the syndrome of partial or, less commonly, complete obstructive jaundice plus the presence of impaired liver function as determined by the galactose tolerance test. It occurs in diffuse liver disease such as cirrhoses, catarrhal jaundice, Weil's disease, and phosphorus or chloroform poisoning. It is the object of most of the special tests of liver function cited in the references on page 144 to aid in segregating this group from uncomplicated partial or complete obstructive jaundice. No test has to date proved successful in making this separation. Until such a test is devised it seems wiser to treat all patients with partial or complete obstructive jaundice as if liver damage were present, namely, with low protein, high carbohydrate diet and administration of bile salts and vitamin K as necessary to maintain normal prothrombin time and as a routine prior to operation.

4 Hematogenous Jaundice—This is characterized by an icterus index above 6, a negative direct van den Bergh test, the presence of urobilinogen in the urine with a negative test for bilirubin in the urine. Bilirubin is present in the duodenal fluid and the feces are usually darker in color than normal and, if quantitative tests are done, will show an increased excretion of urobilinogen. It is differentiated from complete obstructive jaundice by the dark stools and positive tests for urobilinogen in the urine and the absence of bilirubin in the urine. The

dark stools, negative direct van den Bergh test, and absence of bilirubin differentiate it from partial obstructive jaundice. The normal galactose tolerance differentiates it from hepatocellular jaundice. It occurs whenever hemoglobin is destroyed at an excessive rate in the body, therefore, in all forms of internal hemorrhage, intravascular or extravascular hemolysis, hemolytic icterus and malaria, in other words, in all the internal blood destruction groups of anemias and in many instances where internal hemorrhage has not been sufficient to produce anemia. Both hematogenous jaundice and partial or complete obstructive jaundice may be present at the same time, in which case, the laboratory findings will be typical of obstructive rather than hematogenous jaundice. This occurs most frequently in hemolytic icterus which predisposes to formation of bile pigment stones. These may partially or completely occlude the common duct.

5 **Conditions Discussed in Other Chapters**—The blood chemistry changes which may result from vomiting, gastric or duodenal fistulae, diarrhea, deficient absorption of carbohydrate, or starvation should be reviewed at this point. (See Index by Diseases)

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CHAPTER VI

DISORDERS OF THE ERYTHROPOIETIC SYSTEM WITH ESPECIAL REFERENCE TO ANEMIAS AND POLYCYTHEMIAS

I NOMENCLATURE

Much of the difficulty the beginner experiences in hematology is due to the multiplicity of names which have been used for cells of the blood and blood-forming organs, and to the fact that many of these names have not been clearly defined. Some of these names have been used by different investigators for different cells while many different names have been applied to the same cell. In an effort to clarify this nomenclature, Table 6¹ was prepared. In this table the preferred name for each series and cell type is indicated and other names which have been applied to the same cell are given. In the Atlas, in which this table was first published, each cell type is defined and illustrated and its differentiation from other cells which may resemble it is given. All of the names have been used by others except some of those indicated as preferred for the granulocyte series and the erythrocyte series. These new names were not suggested because of the paucity of names but because the names in current use for the same cells have been used with different meanings by different authors. Consequently, if only the old names were used, it would have been necessary to define the meaning of the term each time it was used in order to avoid confusion. Since the sole purpose of a name is to make the meaning clear without repeated definition there seemed no choice other than coming new terms.

In this edition the commonly used term is given in parentheses after the preferred term and usage will decide which term is to be used in subsequent editions. It should be understood that many of the terms in the column marked "Other Names Used" are not exact synonyms but have included the cell as described in the Atlas and defined in this text and other cells which may not even belong to the same series or stage of differentiation.

New names were chosen for cells of the granulocyte (myeloid) series for several reasons. The term "myeloid" means marrow-like while these cells are an integral part of the marrow as are the cells of all the other series. The most important reason is that the term "myeloblast" has been used for all stem cells as well as for the specific stem cell of the granulocyte series. The term "granulocyte series" is already in current use to replace the old term "myeloid." It seems only consistent to substitute the prefix granulo- for myelo- throughout.

¹ Osgood E. E. Histogenesis Classification and Identification of Cells of the Blood and Marrow Based on Cultures and Hematologic Studies of Human Marrow and Blood. *Am J Clin Path* 8: 59-74 (Jan) 1918.

Osgood E. E. and Ashworth Clarence M. Atlas of Hematology. Pp 6-8. J. W. Stacey Inc. San Francisco 1937.

the series and for the characteristic type of leukemia. The term "polymorphonuclear," while widely used, has been used for many years for all stages of the neutrophils, eosinophils and basophils seen in blood and is still so used by many authors. The importance of differentiating clearly the cells with segmented nuclei from the more immature cells has been repeatedly emphasized. The term "lobocyte" is shorter, easier to learn, and limited by definition and derivation to such segmented or lobed cells. In all other series the names of the cells end with the termination "cyte" or for the most immature cells "blast," but the cell which I have named "rhabdocyte" has been called "staff cell," "rod cell," "band cell," and "juvenile cell." None of these terms are accurately descriptive or conform to the terminology of the other cells. The prefix "rhabdo," already used in "rhabdomyoma," means curved rod, stick or wand. It is more accurately descriptive of the shape of the nucleus than "staff" which suggests a straight rod.

The nomenclature of the erythrocyte series differs from the nomenclature of all other series in that the ending "blast" is retained for all cells of the series.

TABLE 6—NOMENCLATURE OF THE CELLS OF THE BLOOD AND BONE MARROW*

Name of series	Recommended name	Names which have been applied to the same cell
Lymphocyte	Lymphoblast	Myeloblast ¹ ; hemocytoblast ² ; lymphoidocyte ³ ; stem cell lymphocyte ⁴
	Prolymphocyte	Large lymphocyte ⁵ ; pathologic large lymphocyte ⁶ ; atypical leukocytoid lymphocyte ⁷ ; monocyte ⁸
	Lymphocyte	Small medium or large lymphocyte; normal lymphocyte; small medium or large mononuclear
Monocyte	Monoblast	Myeloblast ¹ ; hemocytoblast ² ; lymphoidocyte ³ ; lymphocyte ⁴ ; stem cell; immature monocyte
	Promonocyte	Premonocyte ⁹ ; hemohistioblast ¹⁰ ; immature monocyte
	Monocyte	Large mononuclear ¹¹ ; transitional ¹² ; clasmatocyte ¹³ ; endothelial leukocyte ¹⁴ ; histiocyte ¹⁵ ; resting wandering cell ¹⁶
Granulocyte (Myeloid)	Granuloblast	Myeloblast ¹⁷ ; hemocytoblast ² ; lymphoidocyte ³ ; lymphocyte; stem cell
	Progranulocyte 5†	Promyelocyte I ¹⁸ ; myelocyte A ¹⁹ ; myelocyte; non filament ²⁰ class I ²¹
	Progranulocyte A	Promyelocyte II ¹⁸ ; leukoblast ¹ ; basophil myelocyte ²² ; myeloblast ²³ ; premyelocyte ²⁴
	Granulocyte	Myelocyte ²⁵ ; myelocyte B ²⁶ ; non filament ²⁰ class I ²¹
	Metagranulocyte	Metamyelocyte ²⁷ ; juvenile ²⁸ ; myelocyte C ²⁹ ; non filament ²⁰ class I ²¹
	Rhabdocyte	Staff cell; stab cell ³⁰ ; band cell ³¹ ; non filament ²⁰ class I ²¹ ; rod nuclear ³² ; polymorphonuclear
	Lobocyte	Segmented neutrophil ³³ ; polymorphonuclear, filamented ²⁰ class II III IV or V ³⁴

TABLE 6—NOMENCLATURE OF THE CELLS OF THE BLOOD AND BONE MARROW *—(Continued)

Name of series	Recommended name	Names which have been applied to the same cell
Plasmacyte	Plasmablast	Myeloblast ¹ hemocytoblast ² lymphoidocyte ³ lymphocyte ^{4,5} stem cell lymphoblastic plasma cell ¹
	Proplasmacyte	Türk cell ⁶ Türk irritation form lymphoblastic or myeloblastic plasma cell
	Plasmacyte	Plasma cell ⁷ Unna's plasma cell ⁸ Marschalko plasma cell ⁹ plasmacytoid lymphocyte ¹⁰
Erythrocyte	Karyoblast	Megaloblast ¹¹ myeloblast ¹ hemocytoblast ² lymphoidocyte ³ lymphocyte ⁴ stem cell promegaloblast ¹ basophilic normoblast ¹ primitive erythroblast ¹²
	Prokaryocyte	Erythroblast ¹³ megaloblast ¹⁴ orthochromatic normoblast ¹⁵ basophilic normoblast ¹⁶ polychromatophilic normoblast ¹⁷ macro normoblast ¹⁸ macroblast ¹⁹
	Karyocyte	Normoblast ²⁰ pronormoblast ²¹ macronormoblast ²² erythroblast ²³ polychromatophilic normoblast ²⁴
	Metakaryocyte	Normoblast ²⁵
	Reticulocyte	
	Akaryocyte	Erythrocyte red blood cell erythroplastid ²⁶ normocyte ²⁷
Thrombocyte	Megalokaryoblast	Megakaryoblast
	Promegalokaryocyte	Promegakaryocyte
	Megalokaryocyte	Megakaryocyte
	Platelet	Thrombocyte thromboplastid
	Disintegrated cell	Senile cells smudge basket cell smear cell degenerated cell

* Reproduced by permission of the copyright owners from Osgood E. E. and Ashworth Clarence M. Atlas of Hematology. Pp. 255. J. W. Stacey Inc. San Francisco 1937.

† Any basophil from the progranulocyte to the lobocyte is sometimes referred to as a mast cell.

¹ H. Downey and K. Kató

² A. Ferrara

³ A. Pappenheim

⁴ A. A. Maximow and W. Bloom

⁵ An error in classification

⁶ E. E. Osgood

⁷ P. W. Clough

⁸ An obsolete term

⁹ R. Cunningham P. Sabin and C. Doan

¹⁰ Common term for monocytes when found in tissues

¹¹ D. L. Farley H. St. Clair and J. A. Reisinger

¹² W. P. Cooke and E. Ponder

¹³ An error due to interpretation of azurophilic granules as basophilic granules

¹⁴ V. Schilling

¹⁵ A. Pleš

¹⁶ R. B. H. Gradwohl

and not limited to the most immature one. Furthermore, the term "normoblast" is derived from a Latin and Greek root and means "normal stem cell." The cell referred to is neither a normal constituent of the blood nor is it a stem

B Functions of the Red Bone Marrow¹—This constitutes the chief hematopoietic organ throughout postnatal life. Here the red cells, the cells of the granulocyte (myeloid) series and the platelets are formed. In normal adult red marrow the white cells and their precursors are about three times as numerous as the red cells and their precursors, while the megalokaryocytes which are enormous cells with greatly lobulated nuclei and large amount of cytoplasm containing azur granules, form only about 0.2 per cent of the cells present. It is probable that the red cell formation is intravascular (within the sinusoids) and that of the white cells is extravascular. Wright's theory of the formation of platelets by the pinching off of portions of the cytoplasm of the megalokaryocytes which have been extended in pseudopod form into the vascular channels is almost certainly correct.

x Red Cell Formation—In the red cell forming areas, all stages from the earliest karyoblast (megaloblast) to the mature erythrocyte may be found. The earlier cells (see plate I, section IX, and the Atlas for more detailed morphology) have a deeply basophilic cytoplasm containing no hemoglobin and a relatively large nucleus with a reticular chromatin structure and nucleoli. The changes that occur as maturity progresses are loss of the nucleoli, progressive decrease in basophilia of the cytoplasm, progressive increase in the hemoglobin content, progressive decrease in size and a coarsening of the chromatin structure of the nucleus with first aggregation of this into clumps, later assembling of these clumps into a dense pyknotic nucleus in which no structure is visible, and then resorption or extrusion of the nucleus, whole or after fragmentation. Even after the hemoglobin content is normal and the nucleus is gone, basophilic material persists for a time appearing either as polychromatophilia or, with special stains, as the reticulum. Various names karyoblast (megaloblast), prokaryocyte (erythroblast), karyocyte (pronormoblast), metakaryocyte (normoblast), reticulocyte, and akaryocyte (non-nucleated erythrocyte) have been given to different stages in this development. It must be remembered in using these names that they represent merely stages, marked off by more or less arbitrary criteria, in a continuous development and, as many of the changes are independently variable, two cells in the same stage of maturity sometimes have widely different appearances. In the normal marrow of adults, karyoblasts (megaloblasts) are scarce, and the more mature cells (prokaryocytes, karyocytes, metakaryocytes, reticulocytes) are far the more numerous. Only mature akaryocytes (erythrocytes) and a few reticulocytes are allowed to escape into the general circulation. In infancy, more of the immature cells are present in the marrow and reticulocytes are more numerous in the blood. In early embryonic life the karyoblast (megaloblast) is the preponderant cell in erythropoietic zones, and nucleated red cells are numerous in the blood stream. In both marrow and blood, a shift occurs toward more mature forms until at term, nucleated erythrocytes have almost disappeared from the blood.

Increased erythropoiesis is associated with a reversion of the bone marrow and blood picture to a stage resembling that of a less mature individual. The amount of reversion is roughly proportional to the intensity of the erythropoietic stimulus. Thus, in early childhood a much less intense stimulus than is required in later life is adequate to give rise to numerous nucleated erythrocytes in the blood stream.

¹ Sabin Florence R. The Bone Marrow. *Physiol Rev* 8 191-224 (April) 1928

Relationships of the White Cells

The cells below the dotted line are those present in normal blood. The greater the vertical distance of a cell above the dotted line, the greater is its immaturity, and the greater is the stimulus required to introduce it into the general circulation.

Arrows indicate the lines of maturation. The line of descent on the right hand side is that of the lymphocytes. Some hematologists would interchange the positions of the two normal lymphocytes. The middle three lines of descent are those of the granulocytes. The eosinophil and basophil progranulocytes S (promyelocytes I), granulocytes (myelocytes), and metagranulocytes (metamyelocytes) have not been depicted as they differ from the corresponding neutrophil forms only in the nature of the granulation and this is identical with that in the mature eosinophil and basophil forms respectively. The use of a single reproduction to represent the morphologically similar stem cells does not necessarily imply that these cells are actually identical.

This chart should be studied in conjunction with the colored plates of the same cells. It is of particular value for studying the relative sizes of cells and granules as the magnification is close to that usually used in studying such cells.



Stem cell
(Glycoblast or Lymphoblast etc)



Plasma cell
T-lymphocyte form



177u x 155u

Type I



Procytocyte

Type II



Pathological large
lymphocyte



112 x 154u

Monocyte
Large mononuclear
form



Eosinophil
myelocyte



Neutrophil
Myelocyte

Neutrophil
Metamyelocyte



Basophil
myelocyte



Immature normal
lymphocyte



117u x 155u

Normal lymphocyte



Monocyte
Transitional form



155u

Eosinophil
metamyelocyte

Basophil
metamyelocyte

Staphylococcus
(Staphylococcus)



Polymorphonuclear
eosinophil



12u

Normal lymphocyte



u

CHART I

Relationships of the White Cells

The cells below the dotted line are those present in normal blood. The greater the vertical distance of a cell above the dotted line the greater is its immaturity, and the greater is the stimulus required to introduce it into the general circulation.

Arrows indicate the lines of maturation. The line of descent on the right hand side is that of the lymphocytes. Some hematologists would interchange the positions of the two normal lymphocytes. The middle three lines of descent are those of the granulocytes. The eosinophil and basophil progranulocytes S (promyelocytes I) granulocytes (myelocytes) and metagranulocytes (metamyelocytes) have not been depicted as they differ from the corresponding neutrophil forms only in the nature of the granulation and this is identical with that in the mature eosinophil and basophil forms respectively. The use of a single reproduction to represent the morphologically similar stem cells does not necessarily imply that these cells are actually identical.

This chart should be studied in conjunction with the colored plates of the same cells. It is of particular value for studying the relative sizes of cells and granules as the magnification is close to that usually used in studying such cells.



Stem cell
(Myeloblast or Lymphoblast)



Plasma cell
Turk's irritation form



177u x 153u

Type I

Promy leucyte

Type II



Pathological large lymphocyte



12.2 x 15.4u

Monocyte
Large mononuclear form



Eosinophil
myelocyte

Neutrophil
myelocyte



Neutrophil
Metamy leucyte



Basophil
myelocyte

Immature normal lymphocyte



11.6 x 15.3u

Normal lymphocyte



Eosinophil
metamyelocyte

Basophil
metamyelocyte

Staff cell
(Stabkernige)



Polymer of nucleic acid



12

Polytrophonuclear eosinophil



12u

Normal basophil (mast cell)



12u

CHART I

Red Cells (Erythrocytes)

All are shown as stained with Wright's modification of the Romanowsky stain by the technic described in Part Two. The size corresponds to a magnification of 2500 diameters. Hence they may be compared directly with the plates on malaria parasites and white cells.

1 Akaryocyte (normal red cell). Note the round or oval contour and the orange buff color, paler in the center due to the biconcave disc shape. The normal size variation is 6.6 to 8.6 micra but the average size in stained smears is rarely less than 7.0 or more than 8.0 micra and the average is probably about 7.6 micra.

2 Erythrocyte showing achromia. Note the pallor and the increased relative extent of the central pale area. The cell depicted shows the least detectable distinct achromia. More definite degrees of achromia show as pale rings with colorless centers. This is supposed to be due to a decrease in hemoglobin (hence should be called hypochromia as hemoglobin is never completely absent) but the appearance may be simulated by decreased cell diameter. Therefore the saturation index determination is much more reliable for determination of the relative amount of hemoglobin present.

3 Erythrocyte showing punctate basophilia (basophilic stippling) and slight polychromatophilia. Note the blue staining dots called basophilic stippling and the fact that the background can not be described as any shade of orange buff. This cell is at the upper range of normal cell size and cell 5 is at the lower range of normal cell size. Cells larger than this are called macrocytes and smaller than cell 5 are called microcytes.

4 Metakaryocyte (normoblast). Note the dense structureless nucleus (pycnotic) and the moderate polychromatophilia of the cytoplasm. Any cell containing a nucleus which is less than one half the diameter of the cell may be called a metakaryocyte (normoblast). The differentiation from other cells of the erythrocyte series must be made by the pycnotic nuclear structure and a nuclear diameter less than half that of the cell rather than by size or hemoglobin content of the cell. Polychromatophilia is not necessarily associated. The differentiation from leukocytes in those cells of the erythrocyte series which are nucleated and contain no hemoglobin is by the opaque appearance of the cytoplasm.

5 Erythrocyte showing marked polychromatophilia. This cell is at the lower range of normal size. Note that polychromatophilia consists of the combined effects of a decrease in the orange buff staining hemoglobin and an increase in the basophilia (affinity for the blue dye in the stain used) of the stroma of the cell. All possible combinations of these two changes may occur and several of these are illustrated in the cells depicted.

Polychromatophilia may affect red cells showing many other abnormalities, therefore, when present it must be added to other descriptive terms.

6 Reticulocyte. This cell is from a film first stained with the reticulocyte stain and then with Wright's stain as directed in Part Two. Cells showing a blue staining interlacing network with this stain are called reticulocytes. The amount of this network may vary from the smallest fraction of that here shown to a network that fills the entire cell. This may occur in many different types of cell. Like the cell depicted, reticulocytes are often large.

7 Karyocyte (pronormoblast). The nucleus is more than one half and less than two thirds the diameter of the cell which differentiates it from the metakaryocyte (normoblast) and from the prokaryocyte (erythroblast). The sharp contrast between the dark staining basichromatin and light staining oxychromatin may occur also in cells of the plasmacyte series but the individual clumps in cells of the erythrocyte series are smaller and more numerous. Note the extreme basophilia of the cytoplasm which is almost devoid of hemoglobin but still has an opaque appearance which differentiates it from the transparent cytoplasm of all the white cells except those of the plasmacyte series. Many karyocytes (pronormoblasts) contain hemoglobin in the cytoplasm.

8 Prokaryocyte (erythroblast). These are differentiated from karyocytes (pronormoblasts) by the diameter of the nucleus which is more than two thirds the diameter of the cell and from the karyoblast (megaloblast) by the absence of nucleoli. The cytoplasm may be basophilic, polychromatophilic or normochromic but is usually polychromatophilic. In macrocytic anemias hemoglobin is more abundant than in cells of equal immaturity in other diseases.

The karyoblast (megaloblast) is not illustrated. The cytoplasm resembles that of cells 7 and 8 but the nucleus always contains nucleoli and has a structure similar to cell 8 or to the stem cell depicted in plate V. The more immature the karyoblast (megaloblast) the more closely does the nuclear and cytoplasmic structure resemble that of other stem cells but only rarely is difficulty in differentiation from more mature white cells encountered if attention is paid to the details of nuclear structure and the greater opacity of the cytoplasm of the red cell. Large size and polychromatophilia of the cytoplasm are usually but not always present.

In order of increasing maturity (and hence of increasing frequency in the peripheral blood) the cells on this page would be arranged 8, 7, 4, (3, 5, 6), (1). Those in parentheses are at about the same stage of maturity.



76μ

1



75μ

2



88μ

3



78μ

4



66μ

5



111μ X 15μ

6



111 X 133μ

7



166μ X 144μ

8

[LATE 1

Lobocytes and Rhabdocytes (Polymorphonuclear Cells) of Normal Blood

The white cells which may appear in the blood stream are reproduced on this and succeeding pages. All were stained with Wright's stain by the technic given in Part Two. All are drawn to scale corresponding to a magnification of 2500 diameters. Hence they should be held at a distance of 18 inches from the eye to show the detail which is visible with the best microscopes and yet further away to give the appearances of the cells as seen through less perfect optical systems. The apparent and comparative sizes of the cells and granules are best studied in the chart of the relationships of the white cells. The cells are roughly spherical in form and even though flattened in smears this must be kept in mind in studying the films and these illustrations. Thus granules in the cytoplasm between the eye and the nucleus will appear to be in the nucleus etc.

Lobocyte (segmented polymorphonuclear) neutrophil. Note the lobulated structure of the nucleus with its densely staining sharply demarcated interlacing chromatin bands, the pale lilac tint of the cytoplasm and the deeper lilac tint of the diffusely scattered fine uniform neutrophil granules. The quality of this color of the neutrophil granules is one of the best criteria of a satisfactory stain. In understained films these granules may be invisible. These cells may vary in size from 11 to 15 micra. The lobulations of the nuclei may vary from two to five or over, but forms with three or four lobulations are most common.

Rhabdocyte (Staff cell). This differs from the lobocyte (polymorphonuclear neutrophil) only in the form of the nucleus in having a slightly more basophilic cytoplasm and in a tendency to be of slightly larger size (11 to 18 micra). The form of the nucleus is the important point of identification. It is a variously curved or coiled rod or band which may be narrowed in places but never to a filament. The interlacing chromatin network is slightly broader and less sharply demarcated than in the lobocyte (polymorphonuclear). A similar type of nucleus is sometimes noted in cells containing eosinophil or basophil granules.

Eosinophil. Note the uniform large size of the acidophilic granules, which when properly stained have a deep red color with a slight orange cast. Note also that the nuclear structure and the cytoplasmic staining in this cell and in the lobocyte (polymorphonuclear neutrophil) are similar. A bilobed nucleus similar to that here shown is the commonest form and nuclei with over four lobes are uncommon. The number of granules is not infrequently less than in the example shown. The apparent difference in intensity of staining of the granules is due largely to variations in their distance from the surface of the cell nearest the eye. The range of cell size variation is about 11 to 16 micra.

Basophil. Note the marked variation in size of the relatively few granules which stain a deep blue with a slight purplish cast (metachromatic) of varying intensity and character. The cytoplasm tends to be more acidophilic (pink) than that of the eosinophil or neutrophil and the nucleus rarely shows true lobulation but is irregularly shaped and palely and indistinctly stained thus giving the impression of being seen through a haze. As the granules are water soluble some or all may appear as vacuoles in poorly fixed smears. The largest granules are larger than eosinophil granules. The cell size range is from 11 to 15 micra.

Polymorphonuclear
neutrophil



12 u

Staff cell
(Stabkernige)



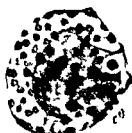
11 x 14 u

Polymorphonuclear
eosinophil



12 u

Normal basophil
(mast cell)



12 u

Mononuclear Cells of Normal Blood

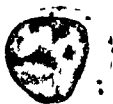
Normal lymphocyte (small lymphocyte small mononuclear S I, S M, or L) Note the round or oval nucleus with coarse indistinctly margined clumps of deeper staining chromatin and the basophilic cytoplasm containing a few azur granules of irregular size and distribution. These cells may vary in size from 7 to 13 micra, but the smaller sizes are most numerous. The nucleus sometimes contains nucleoli and may show deep slit like indentations. Rarely, double nuclei may be observed. The cytoplasm varies greatly in amount from the merest detectable rim to an area equal to that of the nucleus but is usually relatively small in amount. It, also, varies in its degree of basophilia from a very deep to a very pale shade of pure blue. The paler stain is more common when the amount of cytoplasm is relatively large and vice versa. Azur granules are more commonly absent than present. If present, they stain (as always) a color similar to that of the nucleus of the cell containing them. They are irregular in size relatively few in number and tend to aggregate in clumps. They are never diffusely scattered and numerous enough to obscure the color of the cytoplasm.

Immature normal lymphocyte This cell is really a subdivision of the above described group and should not be separated from other normal lymphocytes in differential counting. It is depicted here because it is frequently mistaken for a monocyte and because confusion with the prolymphocyte has resulted from its separation (under the term large lymphocyte) from other normal lymphocytes. In size it does not exceed the largest lobocytes (polymorphonuclears) (12 to 15 micra) while the monocyte and prolymphocyte (pathological large lymphocyte) are definitely larger. Note the relatively large amount of pale blue cytoplasm, the presence of nucleoli in the nucleus and the fact that in all essential details of structure it is merely a variant of the previously described cell. The azur granules are not always present and are never fine and diffusely scattered as in the monocyte. All intermediate stages may be found between the two forms depicted and no diagnostic information is secured by separating them. Some authors believe this form is older rather than younger than the smaller form but the more constant presence of nucleoli in the large forms speaks against this view.

Monocytes (Mon) These were formerly divided into large mononuclears and transitional forms but it has been conclusively shown by the vital staining method that these are all one type of cell. In stained smears, the only apparent difference is in the nuclear form which is round or oval in the 'large mononuclear' and irregular or horseshoe shaped in the transitional. As will be pointed out, these apparent differences in the stained smear are largely if not entirely artefacts. Note for each cell depicted the large size the character of the nuclear structure the relatively large amount of cytoplasm and the very fine diffusely scattered azur granules which it contains. The nuclear chromatin occurs both in clumps and in loosely meshed interlacing strands which give the nucleus a characteristic structure best shown in the 'transitional form' here depicted. The cytoplasm is a clear blue but the azur granules are so fine that they can be seen distinctly only in good stains and with a good microscope. If indistinctly seen, they give the impression that the cytoplasm is of a uniform slightly violet blue with an opacity resembling that of ground glass. This same effect can be secured by holding these illustrations far enough (about 5 feet) from the eye so that the individual granules can no longer be distinguished. If carefully studied with the best stains and microscopes, it will be found that the so called 'large mononuclear' forms do not have round or oval nuclei but have horseshoe or irregularly shaped nuclei which are viewed from such an angle as to appear round. Careful inspection of the 'large mononuclear' here depicted will show that its appearance would be very closely simulated if the transitional form here shown could be viewed as a three dimensional object from a point corresponding to the right hand margin of the page slightly below the level of the cell on the page. The trick of detecting this irregularity of the round nucleus is to survey carefully its margin for a slight acute indentation. From this point a line of demarcation corresponding to the border of an overlapping portion of the nucleus will usually be discernible. In other words the 'large mononuclear' and transitional forms are morphologically identical in fixed as well as in vitally stained preparations and these terms should be discarded and the cells always grouped together under the term monocytes.

¹ Since the frequency with which apparently round cells actually show lobulated or irregularly shaped nuclei was called to my attention by Prof. A. Decastello of the University of Vienna I have been unable to find a normal monocyte which could not be seen by careful examination to have an irregular or horseshoe shaped nucleus.

Normal lymphocyte



10u

Immature normal lymphocyte



117u X 133u

Monocyte
Transitional form



155u

Monocyte
"Large mononuclear
form"



144u

Immature Cells of the Granulocyte (Myeloid) Series

Progranulocytes (promyelocytes) These are cells intermediate in structure between the granuloblast (myeloblast) and the granulocyte (myelocyte). Hence, they may have varied appearances. Note in the progranulocyte S (type I promyelocyte) depicted the persistence of a fine reticulum and nucleoli in the nucleus suggestive of the granuloblast (myeloblast) while the cytoplasm is less basophilic than in the granuloblast (myeloblast), and neutrophil granules have begun to develop similar to those of the granulocyte (myelocyte) stage. Eosinophil or basophil progranulocytes (promyelocytes) of this type can of course also occur. In the progranulocyte A (type II promyelocyte), the nucleus has a coarser chromatin structure without nucleoli like that of the granulocyte (myelocyte) but the cytoplasm is still basophilic and contains only coarse azur granules. The azur granules may be absent but are more often present than in the granuloblast (myeloblast). These cells may vary in size from 11 to 20 micra but the larger sizes are more common. Any cell which has either too mature a nucleus, cytoplasm or granules for a granuloblast (myeloblast) and not sufficiently mature a nucleus, cytoplasm or granules to answer the description of a granulocyte (myelocyte) is most conveniently classed as a progranulocyte (promyelocyte).

Granulocytes (myelocytes) These are of three types according to the nature of the specific granulation (neutrophil, eosinophil or basophil). Note in the neutrophil granulocyte (myelocyte) depicted that the nucleus no longer contains nucleoli; its chromatin structure is relatively coarser than that of the granuloblast (myeloblast); the cytoplasm is neutrophilic or only slightly basophilic and typical neutrophil granules are present. The slightly pinker color of these as compared to those in the lobocyte (polymorphonuclear) shown is due to difference in time of staining and in light intensity, not to differences in the granules. These two cells illustrate the maximum variation in appearance of neutrophil granules in satisfactory stains. If they appear pinker or paler, the staining time has been too short, if bluer or deeper the staining time has been too long. Any cell which has a round or oval nucleus without nucleoli and cytoplasm containing specific granulation is to be classed as a granulocyte (myelocyte). This group is further subdivided according to whether the granules are neutrophil, eosinophil or basophil. Since the eosinophil and basophil granulocytes (myelocytes) differ from the neutrophil only in the character of the granules and since these granules have the same characteristics as those shown and described for the lobocytes (polymorphonuclear), these cells have not been depicted. Size variations from 11 to 20 micra occur but intermediate sizes are most common.

Metagranulocytes (metamyelocytes) These cells are also of three types according to the specific granulation present. As with the granulocytes (myelocytes) only the neutrophil form is shown. These are intermediate between the granulocytes (myelocytes) and rhabdocytes (staff cells) in nuclear structure and form but show cytoplasm and granulations indistinguishable from these cells. Any cell with specific granulations in the cytoplasm which has a nucleus which is not segmented and can not be described as either round or oval nor as a curved or coiled band should be classed as a metagranulocyte (metamyelocyte). The intermediate sizes are more common but these also may vary in size from 11 to 20 micra.

Type I

Promyelocyte

Type II

Neutrophil
MyelocyteNeutrophil
Metamyelocyte

Blast Cells, Prolymphocyte and Proplasmacyte

Blast cell, granuloblast (myeloblast) or lymphoblast Note the deeply basophilic cytoplasm, the relatively large round or oval nucleus which always contains nucleoli and has a finely reticular, almost homogeneous, chromatin structure. The nuclear structure is the important point in identification. The size range is very great (from 8 to 10 micra) but the larger sizes are more common. Unless attention is paid to the internal structure of the nucleus, the smaller forms are apt to be confused with lymphocytes. Azur granules similar to those in the progranulocytes (promyelocytes) are sometimes present in the cytoplasm of the more mature forms. If the majority of other cells in the film are of the granulocyte (myeloid) series these cells may safely be called granuloblasts (myeloblasts) if the majority of the other cells are of the lymphocyte series these cells may safely be called lymphoblasts. Some believe that the lymphoblast differs from the granuloblast (myeloblast) in having a slightly coarser internal nuclear structure and a more dense accumulation of chromatin at the margin of the nucleus giving it a sharper outline. These differences are so slight if they occur as to be unreliable criteria. They probably apply to cells which have begun to take on the character of the prolymphocyte rather than to the most immature cells which appear in acute lymphocytic leukemia. Neither type of cell takes the peroxidase stain hence the differentiation of the type of leukemia must be based on the rest of the blood picture and on the clinical picture. In some cases they will have to be noncommittally reported as blast cells. Monoblasts and the earliest karyoblasts (megakaryoblasts) approach this cell in appearance. This illustration fails to show the clear transparent appearance of the cytoplasm of the granuloblast (myeloblast) which is of value in differentiating it from the karyoblast (megakaryoblast).

Rieder cell Note that this differs from the previously described cell only in the configuration of the nucleus. This is a coiled or curved rod or band rarely even segmented but still showing the internal structure of the granuloblast (myeloblast). The cell depicted is smaller and the nucleus less coiled than is usually the case but all variations described for the blast cell occur.

Prolymphocyte This cell was drawn before accurate criteria for differentiation of the prolymphocytes and lymphocytes were laid down. It is actually a lymphocyte as the diameter does not exceed 15 micra but with this exception the morphology is identical with the prolymphocyte. These cells were formerly called pathological large lymphocytes. Note the large size, relatively small amount of cytoplasm and the nuclear structure intermediate between that of the lymphoblast and the normal lymphocyte. The cell depicted is from the blood of a case of infectious mononucleosis. In this cell the nuclear structure is closer to that of the normal lymphocyte than to that of the lymphoblast. Nucleoli are not infrequently present but the coarser nuclear chromatin differentiates this cell from the stem cell and its larger size (15 to 20 micra definitely larger than the lymphocyte (polymorphonuclear)) and more deeply basophilic cytoplasm differentiates it from even the largest normal lymphocytes. A few coarse azur granules may be present but they are more often absent and never fine and diffusely scattered as in the monocyte. The negative peroxidase stain will differentiate it from the progranulocyte A (type II promyelocyte) which it otherwise resembles.

Proplasmacyte This is often called Turk's irritation form. Note the extreme basophilia of the cytoplasm, the perinuclear paler area in the cytoplasm, the eccentric position of the nucleus, its homogeneous chromatin structure and the presence of nucleoli. The above are the most characteristic features of these cells. They often contain vacuoles but never granules of any type. The mature plasmacyte has the same type of cytoplasm but a coarse cartwheel arrangement of the chromatin in the nucleus. The size variation is 12 to 20 micra for the Turk's form and 8 to 20 micra for other plasma cells.

Stem cell
(Myeloblast or Lymphoblast)



144u

Rieder cell



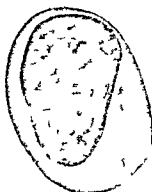
10u

Pathological large
lymphocyte



122 X 15u

Plasma cell
Turks irritation form



177u X 133u

Plasmacyte, Promonocyte and Toxic Neutrophils

Plasmacyte (Marschalko plasma cell) This like the proplasmacyte, has deeply basophilic cytoplasm, an eccentrically placed nucleus, a perinuclear pale area and usually contains no granules. It differs from the proplasmacyte in having a very coarse chromatin structure with sharp demarcations between the dark and pale chromatin. It resembles some of the nucleated erythrocytes very closely but differs in that the individual chromatin clumps are larger. The size varies from 10 to 20 micra in diameter. This cell occurs in small numbers in the blood and marrow normally and may be present in increased numbers in plasma cell leukemia, German measles and multiple myeloma.

Promonocyte This differs from the monocyte in having a finer chromatin meshwork and nucleoli in the nucleus. It differs from the monoblast in having more azurophilic granules in the cytoplasm and in having an irregularly shaped nucleus like the mature monocyte. This cell rarely is found in the blood except in monocytic leukemia.

Toxic neutrophils These two cells illustrate the changes in neutrophil morphology which indicate a grave prognosis. Comparison of these cells with the other neutrophils illustrated will show that the cytoplasm between the granules is more basophilic (bluer staining) that the granules are fewer, larger and bluer staining and that in the cytoplasm of the rhabdocyte (staff cell) there are vacuoles. All degrees of variation in these changes may occur between that shown in these cells and the normal morphology. Note that the metagranulocyte (metamyelocyte) somewhat resembles the monocyte but it is readily differentiated because the other cells on the slide will be rhabdocytes (staff cells) and lobocytes (segmented neutrophils). An increase in monocytes is rarely or never associated with neutrophilic leukocytosis. When changes of the degrees illustrated are present in most of the neutrophils in a slide it is almost certain that the patient will die within a week.

Marschalko plasma
cell



18.0 μ X 18.0 μ

Promonocyte



19.5 μ X 21.0 μ

Neutrophil staff cell
(toxic)



15.0 μ X 15.0 μ

Neutrophil metamyelocyte
(toxic)



19.0 μ X 19.0 μ

Reticulocytes and Platelets

Reticulocytes as stained by the Osgood Wilhelm technic. With this stain the erythrocytes containing no reticulum stain pale green as illustrated by cell 8 and the reticulocytes show varying quantities of blue staining reticulum as illustrated in order of increasing amounts by cells 9, 4, 2, 3, 6, 7, 1, 10, 5 and 11. In a correctly stained preparation there should be no debris between the red cells although the platelets and white cells take a blue stain. If counterstained with Wright's stain the reticulum shows as here illustrated and the remainder of the cell stains as does the akaryocyte (mature erythrocyte) with Wright's stain (Plate I).

Platelets. Normal platelets as stained with Wright's stain in the blood or marrow may show any of the variations in appearance depicted except those in the five largest cells. They often tend to occur in clumps, have a pale blue cytoplasm often with indefinite borders and contain numerous purple staining granules which frequently are clumped toward the center leaving a clear ring of granule free cytoplasm around them. The five large platelets are all abnormal forms which may be seen in the blood in such diseases as purpura hemorrhagica, leukemia, pernicious anemia or myelophthisic anemias. Megakaryocytes (not illustrated) are enormous cells, 30 to 60 micra in diameter, having enormous, greatly lobulated nuclei with a coarsely meshed chromatin network. They never appear whole in the blood but are present in small numbers in normal marrow. They are so much larger than any other marrow cell that they can hardly be mistaken for anything else. Fragments of the nuclei with or without attached cytoplasm may appear in the blood in the rare megakaryocytic leukemias.

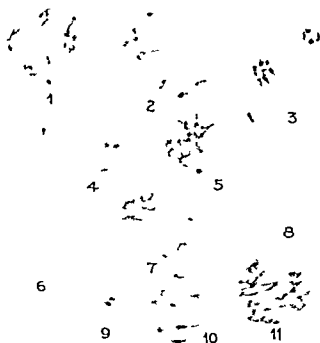


FIGURE 11

The intrinsic factor is not pepsin, rennin or gastric lipase. There is some evidence to suggest that it is formed in the duodenal mucosa as well as in the stomach. The extrinsic factor is found closely associated with vitamin B, but is not a derivative.¹ Some of the vitamins and hormones have also been shown to be necessary for maturation of erythrocytes.

² What Substances Are Necessary for the Formation of Red Cell Stroma and of Hemoglobin?²—Iron is known to be necessary for hemoglobin formation. There is evidence suggesting that copper and certain other metals are also essential, but the reason for this is not yet determined. It seems established that deficiency in stroma building materials can occur, but very little more is known. It has been suggested, but not proved, that the antipernicious anemia principle, which is present in liver and other tissues high in nuclear content, is such a stroma building material.

³ What Is the Lifetime of Erythrocytes in the Blood Stream?—Transfused erythrocytes may persist for 15 to 120 days (average about 80 days), and the rate of urobilinogen excretion gives some idea of the rate at which hemoglobin is destroyed (not necessarily corresponding to the rate of red cell destruction, for some of it may be used over again), but the normal rate of red cell destruction is unknown. Calculations from the rate of erythrocyte regeneration after hemorrhage³ and from the rate of recovery in pernicious anemia,⁴ and from the erythrocyte level maintained by multiple transfusions in aplastic anemia⁵ agree on a duration of life of 30 to 40 days. Relatively little is known of the mechanisms of red cell destruction⁶ in health and disease, but most of it appears to occur in the reticuloendothelial system.

In concluding this resumé I wish to call attention to the fact that the meagre background of fundamental knowledge above outlined is fortunately supplemented by a considerable body of hematologic data established by the method

¹ Strauss, M. B. and Castle W. B. The Nature of the Extrinsic Factor of the Deficiency State in Pernicious Anemia and in Related Macrocytic Anemias. Activation of Yeast Derivatives with Normal Human Gastric Juice. *New England J. Med.* 207: 55-59 (July 14) 1932.

Castle W. B. and Ham T. H. Observations on the Etiologic Relationship of Achylia Gastrica to Pernicious Anemia. V. Further Evidence for the Essential Participation of Extrinsic Factor in Hematopoietic Responses to Mixtures of Beef Muscle and Gastric Juice and to Hog Stomach Mucosa. *J. A. M. A.* 107: 1456-1463 (Oct. 31) 1936.

² Robschelt Robbins F. S. The Regeneration of Hemoglobin and Erythrocytes. *Physiol. Rev.* 9: 666-709 (October) 1929. The numerous articles by Whipple and Robschelt Robbins cited in her review are also very important not only for their contributions to knowledge but as models for the young research worker both in performing and reporting his work. If every individual who undertakes research would first read this series of articles through in chronological order keeping in mind the points outlined in paragraph E of Chapter I of this text, he would derive much inspiration and instruction and medical literature might be spared a few more valueless articles.

³ Schjödtt E. Observations on Blood Regeneration in Man. I. The Rise in Erythrocytes in Patients with Hematemesis or Melena from Peptic Ulcer. *Am. J. M. Sc.* 193: 313-327 (Mar.) 1937.

Schjödtt E. Observations on Blood Regeneration in Man. II. The Influence of Sex Age Form of Hemorrhage Treatment and Complications on Erythrocyte Regeneration After Hematemesis and Melena from Peptic Ulcer. *Am. J. M. Sc.* 193: 327-336 (Mar.) 1937.

⁴ M. C. Riddle personal communication.

⁵ Osgood E. E. Riddle M. C. and Mathews T. J. Aplastic Anemia Treated with Daily Transfusions and Intravenous Marrow. *Case Report. Ann. Int. Med.* 10: 1 (Mar.) 1937.

⁶ Isaacs R. Formation and Destruction of Red Blood Cells. *Physiol. Rev.* 17: 291-303 (Apr.) 1937.

of correlation of clinical and laboratory observations. It is evident, therefore, that hematology is just beginning to emerge from the empiric stage it has been in for so long, toward the status of an applied science. With the hope that this method of presentation will stimulate thinking and research, empiric knowledge is indicated as such, fundamental causes are given when known, and some of the more probably profitable lines of research are suggested.

It is, also, apparent that hematologic findings are not due to disorders of the blood, per se, but are secondary to changes in the blood-forming organs and other tissues in the body. Therefore, the first step in the interpretation of hematologic results is to convert them into a mental picture of what is occurring in these organs and tissues, and the next is to consider the possible causes of such changes.

III THE ROUTINE HEMATOLOGIC EXAMINATION

This is sometimes miscalled "a complete blood count." It consists of a hemoglobin estimation, a red, white, and differential cell count, a sedimentation rate and a color index calculation.

Experience has shown that all patients should have the benefit of this examination, as changes unsuspected from the history and physical examination are thus frequently discovered.

Notwithstanding the great frequency with which these tests are performed, there are few other laboratory procedures in which such gross errors in technic, leading to great inaccuracies in results, are so often made. Therefore, it is highly important that one first make sure that results are based on methods equal in accuracy to those given in Part Two and that they are secured by a person who has mastered the details of the technic and has determined the limits of error of results as there outlined, before proceeding to the interpretation of these results. The interpretations given presuppose that the results are based on satisfactory technic and methods.

*A Normal Values*¹—These differ for the different sex and age groups, so the sex and age of the individual must always be considered in interpreting results. Since blood and plasma volume determinations were not done in conjunction with any of the determinations on which the figures given below are based, it must be recognized that the range of variation includes relative fluctuations due to normal variations in total plasma volume too, and that fluctuations in the total blood stream content of cells or hemoglobin are not accurately represented unless it is permissible to assume that plasma volume alters in such a way as to keep the total blood volume constant.

¹Osgood E. E. Normal Hematologic Standards. Arch Int Med 56: 849-863 (Nov) 1935

Table 7 and figures 3, 4 and 7 summarize the data of an extensive study of normal values. The ninety five per cent range should be learned for each group as a basis for interpretation.

TABLE 7—NORMAL HEMATOLOGIC STANDARDS

	No	Age	Sex	Average	Range 95 per cent
Erythrocyte count	215 259 152	4-13 14-30* 14-30	M & F M F	5.0 5.4 4.8	4.20-5.80 4.60-6.20 4.20-5.40
Hemoglobin per cent	215 259 152	4-13 14-30 14-30	M & F M F	85.0 113.0 100.0	70.0-100.0 100.0-130.0 85.0-115.0
Hemoglobin grams	215 259 152	4-13 14-30 14-30	M & F M F	12.0 15.8 13.8	10.0-14.0 14.0-18.0 11.5-16.0
Hemoglobin coefficient	215 259 152	4-13 14-30 14-30	M & F M F	12.0 14.7 14.3	10.2-13.8 12.8-16.8 12.5-16.0
Cell volume	215 46 63 100 153	4-13 14-17 14-19 18-30 20-30	M & F F M P M	36.0 36.0 41.0 41.0 45.0	31.0-41.0 31.0-41.0 36.0-45.0 36.0-45.0 40.0-50.0
Volume coefficient	304 173 100	4-17 18-30 18-30	M & F M F	36.0 41.0 43.0	31.0-41.0 35.0-45.0 38.0-47.0
Color index	626	4-30	M & F	1.00	0.85-1.15
Volume index	583	4-30	M & F	1.00	0.85-1.15
Saturation index	583	4-30	M & F	1.00	0.90-1.10
Reticulocytes	476	4-30	M & F	1.50	0.50-3.00
Leukocyte count	86 242 269	4-7 8-18 19-30	M & F M & F M & F	10,400 8,400 7,400	5,500-15,500 4,000-13,000 4,500-11,500
Lobocytes (segmented neutrophils)	241 120 216	4-14 15-19 20-30	M & F M & F M & F	38.0 48.0 54.0	16.0-60.0 25.0-70.0 33.0-75.0
Neutrophil rhabdocytes (staff cells)	219 378	4-13 14-30	M & F M & F	3.0 1.0	0.0-10.0 0.0-5.0
Lymphocytes	241 120 236	4-14 15-19 20-30	M & F M & F M & F	48.0 42.0 38.0	21.0-71.0 22.0-62.0 18.0-65.0
Monocytes	219 378	4-13 14-30	M & F M & F	3.0 4.0	1.0-7.0 0.0-9.0
Eosinophils	219 378	4-13 14-30	M & F M & F	2.8 3.0	0.0-8.0 0.0-6.0
Basophils	597	4-30	M & F	0.5	0.0-2.0
Disintegrating cells	219 378	4-13 14-30	M & F M & F	5.0 3.0	0.0-10.0 0.0-7.0
Sedimentation rate 15 min 45 min	853 853	4-30 4-30	M & F		0.0-5 1.0-30

* When thirty is the upper age limit given values few older persons were included in the series on which

above this

B The Unavoidable Error in determinations which involve the counting

2¹—In
a fro

a larger sample, there exist errors due to chance which are entirely independent of the errors in calibration of apparatus or in technic. It is important that physicians and students know of these errors in order that they may interpret red, white and spinal fluid cell counts correctly. Table 8 has been prepared to aid in interpretation by giving the error in percentage directly from the number of cells counted.

TABLE 8—THE UNAVOIDABLE ERROR IN COUNTING METHODS

No. of cells counted \backslash	Standard deviation \pm^*	Per cent within which true count lies $\dagger \pm$	Standard deviation of the difference $\ddagger \pm$	Significant difference in per cent $\S \pm$
10	3.1	62	4.5	90
20	4.5	45	6.3	63
30	5.5	37	7.7	51
40	6.3	32	8.9	45
50	7.0	28	10.0	40
60	7.7	26	10.9	36
70	8.4	24	11.8	34
80	8.9	22	12.6	32
90	9.5	21	13.4	30
100	10.0	20	14.1	28
125	11.2	18	15.8	25
150	12.2	16	17.3	23
175	13.2	15	18.7	21
200	14.1	14	20.0	20
250	15.8	13	22.4	18
300	17.3	12	24.5	16
350	18.7	11	26.4	15
400	20.0	10	28.3	14
500	22.4	9	31.6	13
600	24.5	8	34.6	12
800	28.3	7	40.0	10
1200	34.6	6	49.0	8

* \sqrt{x} $\dagger \frac{2\sqrt{x}}{x} \quad 100$ $\ddagger \sqrt{2x}$ $\S \frac{2\sqrt{2x}}{x} \quad 100$

In counting methods, it has been shown mathematically that 95 per cent of results will be included in a range of plus or minus two standard deviations or plus or minus three probable errors, from the average, and that only 5 per cent of results or 1 in 20 will be excluded. This range which includes 95 per cent of results is satisfactory to use in interpretation of laboratory data. The standard deviation¹ (σ) has

¹ Tables showing the frequency with which a result differing by any number of standard deviations from the average will occur in an infinite series are given in books on statistics.

been shown to equal the square root of the number of cells counted. For example, if a red cell count of 40 million is based on a count of 400 cells in the counting chamber the standard deviation equals the square root of 400 or 20 and two standard deviations equals 40. Therefore, there is only one chance in 20 that the actual count of that blood is less than 36 or more than 44 million.

To simplify the calculation, Table 8 gives the error in percentage of the number of cells counted. In the above example, opposite the number of cells counted, 400, is ± 10 per cent. Ten per cent of 400 is 40 or two standard deviations. Therefore, there is only one chance in 20 that the actual count differs by more than plus or minus 10 per cent from the count based on 400 cells, or is less than 36 or more than 44 million.

To determine the significance of a difference between two results, statisticians use the formula $\sqrt{\sigma_1^2 + \sigma_2^2}$, when σ_1 equals the standard deviation of one result and σ_2 equals the standard deviation of the second result. In laboratory work, if two counts differ by more than twice the standard deviation of the difference there is only one chance in twenty that the difference in these counts is due to chance alone. To save the necessity of doing the mathematics, column 4 in Table 8 has been prepared which shows in percentage the approximate difference that must occur to be significant. Use of this column may be illustrated as follows: a red cell count of 20 million per c. mm., based on a count of 400 cells, is obtained, and one week later the count is 22 million, based on a count of 440 cells. Is the count increasing? In column 4 opposite 400 cells is found ± 14.0 per cent and 14.0 per cent of 20 million is 280,000. The difference between the counts is only 200,000. This difference, being less than the significant difference of 280,000 might well be due to chance alone and not actually to an increase in the count.

From this table it can be seen that the greater the number of cells counted the smaller the unavoidable error will be. Thus explains why it is better to draw the blood up to the 1 mark or count more than the usual number of squares if counts are low.

IV. NUMBER, VOLUME AND HEMOGLOBIN CONTENT OF ERYTHROCYTES

A Normals—1. Red Cell Count¹—(a) *Males Over 14*—In 259 healthy men between the ages of 14 and 30 years an average of 5.4

¹Osgood E. E. Hemoglobin Color Index, Saturation Index and Volume Index Standards (Based on the findings in 137 Young Men) Arch Int Med 37: 685-706 (May) 1926.

Osgood E. E. and Haskins, H. D. Relation between Cell Count Cell Volume and

million erythrocytes per c mm was found with extremes of 4.4 and 6.4 million per c mm. Ninety-five per cent of the results were rather evenly distributed between 4.6 and 6.2 million. These results have been confirmed.¹ Presumably the figures for older men are the same, but this remains to be determined. Most texts still give the average normal as 5.0 million per c mm, a figure based on work done long ago by obsolete methods on a very few individuals and since copied from one text to another without proper confirmation.

(b) *Females Over 14*—In 152 healthy women between the ages of 14 and 30 years, an average of 4.8 million per c mm was found, with extremes of 4.0 and 5.8 million. Ninety-five per cent of the results were between 4.2 and 5.4 million per c mm. This work has also been confirmed. These figures are clinically satisfactory for older women. The same criticism applies to the figure of 4.5 million given in most texts as to the figure of 5.0 million for men. Undoubtedly these errors would have been corrected sooner if they had fallen outside of the range of normal.

(c) *Childhood*²—In a study of 215 children from 4 to 13 years of age the average red cell count was 5.0 million, with extremes of 4.0 to 6.0 million and 95 per cent of the results between 4.2 and 5.8 million. No sex difference was observed. Mugrage and Andresen³ found an average red cell count of about 4.4 million in this age period with a 95 per cent range of 4.0 to 5.0 million. It is probable that the correct average for this group lies somewhere between the values of 4.4 million found by Mugrage and Andresen and 5.0 million found in our study.

(d) *Infants*—Mugrage and Andresen³ found a decrease in red cell count to about 4.2 million during the first 2 months, 3.9 million at 2 to 4 months, and about 4.3 million from 4 months to 4 years, with most of the counts in this age period falling between 3.8 and 4.8 million.

Hemoglobin Content of Venous Blood of Normal Young Women Arch Int Med 39 643-655 (Mar) 1927

¹ Wintrobe M M Blood of Normal Men and Women Erythrocyte Counts Hemoglobin and Volume of Packed Red Cells of 229 Individuals Bull Johns Hopkins Hosp 53 118-130 (Sept) 1933

Andresen Marjory I and Mugrage E R Red Blood Cell Values for Normal Men and Women Arch Int Med 58 136-146 (July) 1936

McGeorge M Haematological Variations in Fifty Normal Adult Males J Path & Bact 42 67-73 (Jan) 1936

Mugrage E R and Andresen Marjory I Red Blood Cell Values in Adolescence Am J Dis Children 56 997-1003 (Nov) 1938

² Osmond E E and Baker R L Erythrocyte Hemoglobin Cell Volume and Color Volume and Saturation Index Standards for Normal Children of School Age Am J Dis Child 50 343-353 (Aug) 1935

³ Mugrage E R and Andresen Marjory I Values for Red Blood Cells of Average Infants and Children Am J Dis Child 51 775-791 (Apr) 1936

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¹Osgood E. E. Hemoglobin Color Index Saturation Index and Volume Index Standards (Based on the Findings in 137 Young Men) Arch Int Med 37 685-706 (May) 1926

Osgood E. E. and Haskins H. D. Relation between Cell Count Cell Volume and

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(b) *Females Over 14*—In 152 healthy women between the ages of 14 and 30 years, an average of 4.8 million per c mm was found, with extremes of 4.0 and 5.8 million. Ninety five per cent of the results were between 4.2 and 5.4 million per c mm. This work has also been confirmed. These figures are clinically satisfactory for older women. The same criticism applies to the figure of 4.5 million given in most texts as to the figure of 5.0 million for men. Undoubtedly these errors would have been corrected sooner if they had fallen outside of the range of normal.

(c) *Childhood*²—In a study of 215 children from 4 to 13 years of age the average red cell count was 5.0 million, with extremes of 4.0 to 6.0 million and 95 per cent of the results between 4.2 and 5.8 million. No sex difference was observed. Mugrage and Andresen³ found an average red cell count of about 4.4 million in this age period, with a 95 per cent range of 4.0 to 5.0 million. It is probable that the correct average for this group lies somewhere between the values of 4.4 million found by Mugrage and Andresen and 5.0 million found in our study.

(d) *Infants*—Mugrage and Andresen³ found a decrease in red cell count to about 4.2 million during the first 2 months, 3.9 million at 2 to 4 months, and about 4.3 million from 4 months to 4 years, with most of the counts in this age period falling between 3.8 and 4.8 million.

Hemoglobin Content of Venous Blood of Normal Young Women Arch Int Med 39 643-655 (May) 1927

¹ Wintrobe M M. Blood of Normal Men and Women. Erythrocyte Counts, Hemoglobin and Volume of Packed Red Cells of 229 Individuals. Bull Johns Hopkins Hosp 53 118-130 (Sept) 1933

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McGeorge M. Haematological Variations in Fifty Normal Adult Males. J Path & Bact 42 67-73 (Jan) 1936

Mugrage E R and Andresen Marjory I. Red Blood Cell Values in Adolescence. Am J Dis Children 56 997-1003 (Nov) 1938

² Osgood E E and Baker R L. Erythrocyte Hemoglobin Cell Volume and Color Volume and Saturation Index Standards for Normal Children of School Age. Am J Dis Child 50 343-358 (Aug) 1935

³ Mugrage E R and Andresen Marjory I. Values for Red Blood Cells of Average Infants and Children. Am J Dis Child 51 775-791 (Apr) 1936

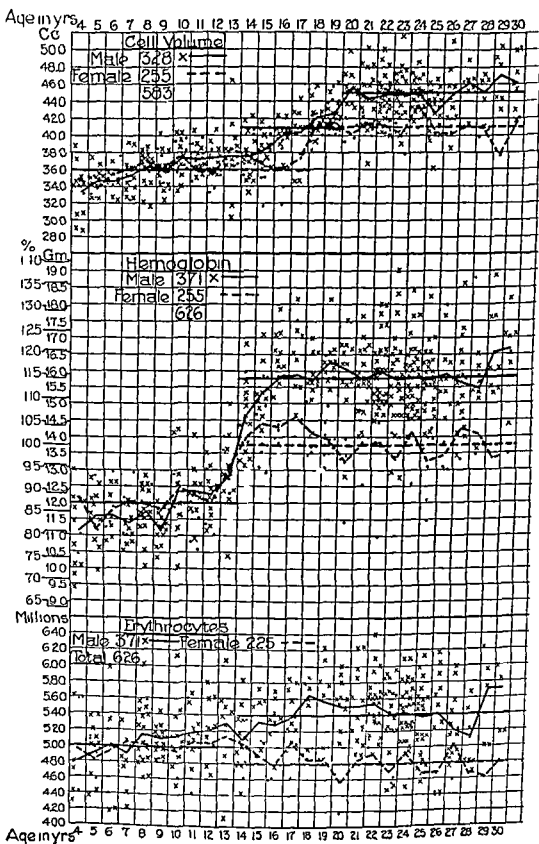


FIG 3—Variations in erythrocyte count hemoglobin and cell volume with age and sex

(e) *Newborn*¹—The average red cell count in newborn of both sexes is about 4.6 million during the first 10 days with 95 per cent of the results falling between 3.5 and 5.5 million. These figures are based on a study of 200 infants,² about 10 males and 10 females being studied on each day. In all of these, the cord was clamped immediately. B. I. Phillips³ has shown that by varying the time of clamping the cord one can alter the red cell count (taken 20 to 30 hours after birth) by about 1 million cells. Thus, in 37 infants in whom the cord was clamped at once the average red cell count was 4.9 million, while in 33 infants in whom the cord was not clamped until after the uterus had firmly contracted, the average count was 6.06 million. This doubtless accounts for the extraordinary variations in the results reported in the literature.

2 **Hemoglobin⁴ and Blood Iron** (Fig. 3)—All hemoglobin estimations should be reported in grams per 100 cc. and the method used should be stated. Otherwise the great variation (13.8 to 17.3) in the number of grams of hemoglobin taken as 100 per cent in different methods and the enormous differences in the accuracy of the methods will make correct interpretation of the results impossible. Most hemoglobin figures in the literature are valueless, either because a grossly inaccurate method such as the Dare or Tallqvist, has been used or because the method and the figure for the grams of hemoglobin corresponding to 100 per cent on the particular instrument are not given. There is no logical basis for reporting the figures in per cent, because the normal hemoglobin differs in the two sexes and in the various age groups. All interpretations given below presuppose that an accurate method has been used. Results are reported in grams per 100 cc. with percentage figures, corresponding to 13.8 grams per 100 cc. as 100 per cent, in parentheses.

¹ Allumbaugh H. R. A Study of the Blood of the Mother and New Born. *Proc. Soc. Exp. Biol. and Med.* 26: 814-816 (June) 1929.

Dhar J. Haematological Studies of Fifty New Born. *Indian J. Ped.* 1: 249-267 (July) 1934.

² Chuinard E. G., Osgood E. E. and Ellis Dorothy M. Erythrocyte Counts, Hemoglobin, Cell Volume and Color, Volume and Saturation Index Standards for Healthy Newborn Infants. To be published.

³ Personal communication.

⁴ Williamson C. S. Influence of Age and Sex on Hemoglobin. *Arch. Int. Med.* 18: 505-528 (Oct.) 1916. This is the largest series of hemoglobin estimations that has ever been reported and the results have great relative accuracy but as has been pointed out (Drucker P. Investigations on the Normal Values for Hemoglobin and Cell Volume in the Small Child. *Acta Paediat.* 3: 1-56 1923) there is reason to believe that all the results are uniformly high. Unfortunately no red cell counts were reported. Other references on hemoglobin are the same as those given for red cells for the corresponding age and sex groups.

Jenkins C. E. and Don C. S. D. The Haemoglobin Concentration of Normal English Males and Females. *J. of Hygiene* 33: 36-41 (Jan.) 1933.

⁵ There are on the market at least four entirely different standards for the Sahli type of hemoglobinometer alone. With these 13.8 gm., 15.0 gm., 15.6 gm. and 17.3 gm. of hemoglobin are supposed to correspond to 100 per cent.

after them for the convenience of those who have become accustomed to thinking in terms of per cent

A number of articles have appeared on the normal *blood iron* estimation¹ Investigators disagree as to how accurately blood iron determinations correspond to hemoglobin determinations² Except in patients receiving iron therapy or when hemoglobin is being rapidly destroyed, the plasma iron is relatively constant at 0.4 to 0.7 mg per 100 cc being, as a rule, less than 2 per cent of the total blood iron, so that for practical purposes, the blood iron estimation is simply another way of determining hemoglobin The hemoglobin determination is much simpler and, therefore, except in research studies, is all that is necessary Since hemoglobin contains 0.335 per cent iron, the normal values for blood iron may be determined from the hemoglobin values given by the use of this factor

(a) *Males Over 14*—In 259 healthy adult males an average of 15.8 grams per 100 cc (114.5 per cent) was found with extremes of 13.0 grams (95 per cent) and 19.0 grams (138 per cent) Ninety five per cent of the results fell between 14.0 grams (102 per cent) and 18.0 grams (130 per cent)

(b) *Females Over 14*—In 152 healthy women the average was 13.8 grams per 100 cc (100 per cent) with extremes of 11.0 grams (80 per cent) and 16.5 grams (120 per cent) Ninety five per cent of the results fell between 11.5 grams (85 per cent) and 16.0 grams (115 per cent)

(c) *Children*—A study of 215 children from 4 to 13 years of age revealed an average of 12.0 grams (87 per cent) with extremes of 9.4 grams (68 per cent) to 14.9 grams (108 per cent) and a range of 10 grams (70 per cent) to 14 grams (100 per cent) to include 95 per cent of the cases No significant sex differences were observed Mugrage and Andresen³ found an average of about 13.5 grams (98 per cent) with a 95 per cent range of results from 11.5 grams (83 per cent) to 15.0 grams (109 per cent) for the same age period The correct result will probably prove to lie somewhere between the two averages of 12.0 grams (87 per cent) and 13.5 grams (98 per cent)

(d) *Infants*⁴—Mugrage and Andresen⁵ found a rapid fall from an average of 17.1 grams (124 per cent) at birth to 14.6 grams (106 per

¹ Haden R L The Determination of Hemoglobin by the Iron Content Method J Lab and Clin Med 19 406 (Jan) 1934

² Jenkins C E and Thomson M L The Distribution of Iron in Blood Brit J Exper Path 18 175-190 (June) 1937

³ See reference page 175

⁴ Mackay Helen M M The Normal Haemoglobin Level During the First Year of Life Revised Figures Arch Child 8 221-225 (June) 1933

⁵ Elvehjem C A Peterson W H and Mendenhall Dorothy Reed Hemoglobin Content of the Blood of Infants Am J Dis Child 45 105-112 (July) 1933

⁶ See references page 175

cent) at 3 days to 2 months, and 11.1 grams (81 per cent) at 2 to 4 months. From 4 months to 2 years the hemoglobin averaged about 12.0 grams (87 per cent), then increased to a level of 13.3 grams (96 per cent) for the period from 2 to 4 years. The 95 per cent range of results in this age period from 2 to 4 years is between 10 grams (73 per cent) and 14 grams (102 per cent) per 100 cc.

(e) *Newborn*—On the first 4 days of life the hemoglobin averages about 17.2 grams (125 per cent) per 100 cc, with a drop thereafter to about 16.0 grams (116 per cent) by the tenth day. The average for the 10 day period is 16.3 grams (118 per cent), with a range including 95 per cent of the results of 14.0 grams (101 per cent) to 20.0 grams (145 per cent). These results are based on a study of 200 infants, about 10 males and 10 females being studied on each day. In these newborn infants the cord was clamped immediately after delivery. In 38 newborn infants in whom the cord was clamped at once, B. I. Phillips found an average of 15.6 grams per 100 cc (113 per cent) with a range of 12.3 grams (89 per cent) to 18.1 grams (131 per cent), while in 33 in whom the cord was not clamped until after the uterus had firmly contracted, the average was 19.3 grams (140 per cent) and the range was 17.2 grams (125 per cent) to 21.9 grams (159 per cent).

3 Hemoglobin Coefficient, Color Index and Corpuscular Hemoglobin (Fig. 4)—To calculate the color index, one needs not only an accurate red cell count and hemoglobin estimation on the patient's blood, but also a normal standard for comparison. This standard is the average number of grams of hemoglobin per 100 cc of blood calculated to a count of 5.0 million red cells per c. mm. in the average healthy person of the same sex and in the same age group as the patient. I have introduced the term *hemoglobin coefficient* to replace the awkward expression, "the number of grams of hemoglobin per 100 cc of blood calculated to a red cell count of 5.0 million per c. mm."

The corpuscular hemoglobin of Wintrobe¹ is the average hemoglobin content of one red cell in micromicrograms and may be determined by multiplying the hemoglobin coefficient by 2.0 or dividing the patient's hemoglobin in grams per 100 cc by the red cell count in millions per c. mm. and multiplying by 10². This gives the same information as the color index, but is less satisfactory for clinical use since a separate set of normals has to be remembered for each age and sex.

¹ Wintrobe M. M. Size and Hemoglobin Content of the Erythrocyte. J. Lab. and Clin. Med. 17: 899 (June) 1932.

²
$$\frac{1,000,000,000 \text{ micromicrograms per } 1 \text{ gm}}{5,000,000 \text{ r. b. c. per c. mm.} \times 100,000 \text{ c. mm. per } 100 \text{ cc.}} = 2.0$$

The color index expresses the ratio between the amount of hemoglobin per unit number of red cells in the patient's blood and the amount of hemoglobin per unit number of cells in the blood of the average normal individual of the patient's age and sex. It is calculated by dividing the per cent of hemoglobin by the per cent of red cells. For this calculation, the normal hemoglobin coefficient for the patient's age

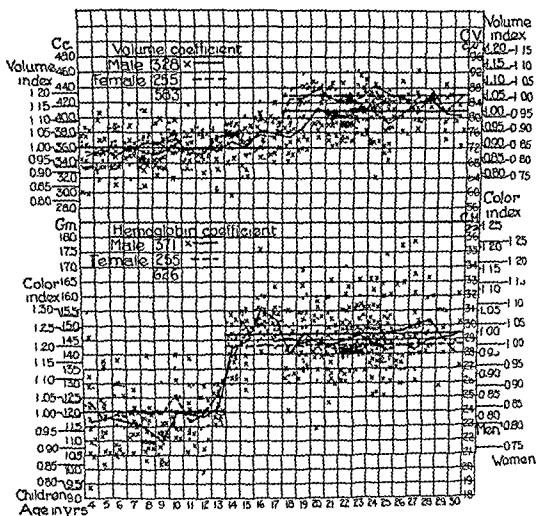


FIG. 4.—Variations in hemoglobin coefficient, color index and corpuscular hemoglobin and in volume coefficient, volume index and corpuscular volume with age and sex.

and sex is considered 100 per cent and a red cell count of 50 million is considered as 100 per cent. It may also be determined by dividing the patient's hemoglobin coefficient by the average normal hemoglobin coefficient or the patient's corpuscular hemoglobin by the average normal corpuscular hemoglobin.

Calculating the average hemoglobin figures per 100 cc. to a red cell count of 50 million for the different groups gives the following as the hemoglobin coefficients and corpuscular hemoglobins.

(a) *Males Over 14*—The hemoglobin coefficient is 14.7 grams and the corpuscular hemoglobin is 29.4 micromicrograms

(b) *Females Over 14*—The hemoglobin coefficient is 14.3 grams and the corpuscular hemoglobin is 28.6 micromicrograms

(c) *Children 4 to 13*—The hemoglobin coefficient is 12.0 grams and the corpuscular hemoglobin is 24.0 micromicrograms

(d) *Infants 10 Days to 4 Years*—The hemoglobin coefficient is 14 grams and the corpuscular hemoglobin is 28.0 micromicrograms

(e) *Newborn Infants*—The hemoglobin coefficient is 16.0 grams and the corpuscular hemoglobin is 32.0 micromicrograms

The above hemoglobin coefficient figures must be used as 100 per cent hemoglobin in calculating color indexes if the results are to be of clinical value. Normal color indexes for all groups average 1.0 and the range is 0.85 to 1.15. Results below 0.80 or over 1.20 are to be regarded as definitely pathologic. To be of value the color index must be based on red cell counts and hemoglobin estimations in which the maximum error is not over 10 per cent. Hence, color indexes based on the Tallqvist or Dare hemoglobin methods are more apt to be misleading than helpful. Tables and a chart greatly simplifying this calculation are given on pages 487 to 495.

4 **Red Cell Volume Determination**¹ (Fig. 3)—This and the index calculations to follow, while not a part of the routine blood examination, are considered here because they are always done in conjunction with a red cell count and hemoglobin estimation. They should be determined in all cases of anemia.

The normal volumes of packed red cells per 100 cc. of blood for the different groups as determined on venous blood containing 2 mg. of potassium oxalate per cc. by the technic recommended on page 461 are given below.

(a) *Adult Males*—One hundred and fifty-three men averaged 45 cc. The extremes were 36 cc. and 52 cc. Ninety-five per cent of the results were between 40 and 50 cc.

(b) *Adult Females and Adolescent Males*—One hundred and six females, 18 to 30 years of age, and 63 males, 14 to 19 years of age averaged 41 cc. The extremes were 33 cc. and 46 cc. Ninety-five per cent of the results were between 36 and 45 cc.

(c) *Children and Adolescent Females*—A study of 215 children, 4 to 13 years of age, and 46 females, 14 to 17 years of age, showed an average of 36 cc., with extremes of 29 to 46 cc. and ninety-five per cent of the

¹ For references see pages 14-17.

results between 31 and 41 cc. Mugrage and Andresen¹ found an average normal cell volume for children of this age group of about 38 cc.

(d) *Infants*—Mugrage and Andresen² found a rapid drop in cell volume from an average of 50 cc at birth to 41 cc at 3 days to 2 months, and 32 cc at 2 to 4 months. From 4 months to 4 years the average was about 36 cc per 100 cc with a 95 per cent range in this age period of 32 to 40 cc per 100 cc.

(e) *Newborn Infants*—The cell volume in the first 3 days averages about 46 cc of packed cells per 100 cc of blood and then falls to about 40 cc by the tenth day. The average for the first 10 days is 44 cc with a 95 per cent range of 35 to 55 cc based on a study of 200 infants, about 10 of each sex being studied on each day.

5 Volume Coefficients, Volume Index and Corpuscular Volume³ (Fig. 4)—The volume coefficient for a given sex and age group is the average cc of packed red cells per 100 cc of blood calculated to a red cell count of 50 million per c mm in normal individuals in that group.

The corpuscular volume of Wintrobe⁴ may be determined by multiplying the volume coefficient by 20 or by dividing the cell volume in cc per 100 cc by the red cell count in millions per c mm and multiplying by 10. The corpuscular volume is the average volume of one red cell in cubic microns. It gives the same information as the volume index, but is less satisfactory for clinical purposes because the values for different age and sex groups are different.

The volume coefficients and corpuscular volumes for the different groups, as calculated for cell volume determinations on oxalated blood, are as follows:

(a) *Males Over 18*—The average normal volume coefficient is 41 cc (54.5 \pm 4.5, \bar{x} = 41). The average corpuscular volume is 82 cubic micra.

(b) *Females Over 18*—The average normal volume coefficient is 43 cc (48.5 \pm 4.1, \bar{x} = 43). The average corpuscular volume is 86 cubic micra.

The reason why the erythrocytes of women are slightly but significantly larger than those of men has not yet been satisfactorily explained.

(c) *Children and Adolescents 14 to 17 Years of Age*—The average normal volume coefficient is 36 cc (Children—50.5 \pm 3.6, \bar{x} = 36,

¹ See reference page 175.

² See reference page 175. The correction of 6.4 per cent for shrinkage which they added has been subtracted to make the results comparable with figures obtained using oxalate.

³ For references see pages 171-179.

⁴ Wintrobe M. M. *Size and Hemoglobin Content of the Erythrocyte*. J. Lab. and Clin. Med. 17: 899 (June) 1932.

Adolescent Males—54 50 41 \bar{x} , \bar{x} = 37, Adolescent Females—48 50 36 \bar{x} , \bar{x} = 37) and the corpuscular volume is 72

(d) *Infants*—The average volume coefficient calculated from the data of Mugrage and Andresen after correction to use of oxalated blood is 42 (43 5 36 \bar{x} , \bar{x} = 42) and the corpuscular volume is 84

(e) *Newborn Infants*—In newborn infants during the first 10 days of life the average volume coefficient is 48 cc (46 5 44 \bar{x} , \bar{x} = 48), and the corpuscular volume is 96 cubic micra

The normal red cells are distinctly larger in the period shortly after birth than at other age periods

The volume index is the per cent of cell volume divided by the per cent of red cells when the volume coefficient for the patient's sex and age group is taken as 100 per cent cell volume and 50 million cells per c mm as 100 per cent cells. See pages 487 to 495 for table and chart simplifying the calculation. The volume index expresses the ratio of the mean size of the erythrocytes in the blood examined to the mean size of the erythrocytes in the average blood of normal individuals of the patient's sex and age group. In all groups the normal average is 10, with a range of 0.85 to 1.15 and results under 0.80 and over 1.20 are to be regarded as pathologic

6 Saturation Index and Corpuscular Hemoglobin Concentration—The saturation index is the per cent of hemoglobin divided by the per cent volume, when the hemoglobin coefficient and volume coefficient for the patient's sex and age group, respectively, are taken as 100 per cent hemoglobin and 100 per cent cell volume. See page 492 for simplified calculation. It expresses the ratio between the hemoglobin per unit volume of cells in the blood examined and the average hemoglobin per unit volume of cells in the blood of healthy persons of the same sex and in the same age group. The average normal figure is 10 with a range of 0.85 to 1.15, and results under 0.80 or over 1.20 should be regarded as pathologic

Wintrobe's corpuscular hemoglobin concentration¹ may be calculated in per cent by dividing the subject's grams of hemoglobin per 100 cc by the subject's cc of packed red cells per 100 cc and multiplying by 100. The average for all ages and both sexes is 34 per cent, with a range of 29 to 39 per cent. It gives the same information as the saturation index and may be calculated from the saturation index by multiplying by 34

7 Morphology of Erythrocytes—This should always be studied. The morphology and staining of the akaryocyte (normal erythrocyte)

¹Wintrobe M M. Size and Hemoglobin Content of the Erythrocyte. J Lab and Clin Med 17: 899 (June) 1932

are portrayed in Plate I and described in the accompanying legend. A few cells as large as 9 micra or as small as 6 micra in diameter, or an occasional cell showing slight polychromatophilia or poikilocytosis can not be considered abnormal.

A few nucleated erythrocytes are present in the blood at birth, but these rapidly decrease in number and are seldom found after the fifth day, although study of a sufficient number of slides will reveal the fact that an occasional metakaryocyte (normoblast) may be found in the blood of most normal persons.

B Physiologic Variations¹⁻¹ **Red Cells and Hemoglobin**—Most of the work so far reported has been done by inaccurate methods, hence it requires confirmation. The hemoglobin variations correspond roughly to those in the red cell count.

(a) *Nutritional State*—The count is said to be higher in thin or muscular, than in stout individuals. Heavy meals and especially large fluid intake cause a temporary slight decrease due to increased plasma volume. During hunger periods there is an increase. A 24 hour fast is said to result in a rise of 500,000 red blood cells per c mm probably due to decreased plasma volume.

(b) *Climate and Race*²—In winter there is said to be about 500,000 more red cells than in summer. No significant seasonal variations were noted in our series, but the seasonal differences in climatic conditions in Portland are not extreme. Change of residence from temperate to tropical zones is said to cause a drop of 500,000 to 2,000,000 in the red cell count but this is, in all probability, due to the frequency of contracting malaria. Wintrobe³ found no significant differences in erythrocyte counts in the subtropical climate of New Orleans from those we reported for Portland, Oregon. Data from other parts of the world are now available which indicate that there are no significant differences in normal values with race or climate. A few large series are reported with low red cell counts and with hemoglobin determinations which agree with those given in this text. In all of these Hayem's solution, which has been shown to give low counts, was used as a diluting fluid.

(c) *Pregnancy*⁴—During pregnancy, a gradual fall in hemoglobin, red cell count, and cell volume occurs, which reaches a level of about 15

¹ Wintrobe M. M. The Erythrocyte in Man. *Medicine* 9: 195-235 (May) 1930.

² Sokhey S. S., Gokhale S. K., Malandkar, M. A. and Billimoria H. S. Red Cells, Haemoglobin, Colour Index, Saturation Index and Volume Index Standards. Part I. Normal Indian Men. A Study Based on the Examination of 121 Men. *Ind Jour Med* 25: 505-528 (Oct) 1937. Part II. Normal Indian Women. A Study Based on the Examination of 101 Women. *Ibid* 25: 723-739 (Jan) 1938.

³ See references under normal red cell count.

⁴ See references in Chapter XI.

per cent below the average normal for all three by the sixteenth to twenty second week. The values remain at this level until about the thirty fourth week when a sharp rise of about 10 per cent occurs, followed immediately by a further fall to the previous level for red cell count and cell volume, and to a still lower level for hemoglobin probably due to iron storage in the liver of the fetus. At term the hemoglobin averages about 11.5 grams, or between 15 and 20 per cent below the normal average. Following normal delivery a further fall of 5 per cent occurs with a gradual return to normal, first of red cell count, then of cell volume and finally of hemoglobin. The latter does not reach full normal until about 6 months post partum. At least part of the apparent decrease is due to the increased plasma volume of pregnancy.

These physiologic variations are to be sharply distinguished from the rare severe anemia of pregnancy which simulates pernicious anemia in all respects, except that spontaneous recovery occurs after the uterus is emptied, and from other anemias which may complicate pregnancy.

(d) *Altitude*¹—The red cell count increases about 50,000 to 100,000 cells per 1000 feet and the cell volume rises correspondingly but the hemoglobin is not greatly elevated. In other words, the cells have a normal volume index, but low color and saturation indexes. Both new cell formation, as indicated by increased reticulocyte count, and redistribution of cells in the circulation seem to contribute to this increase. The specific stimulus has been shown to be the decreased oxygen saturation of the blood.

(e) *Drugs and Therapeutic Measures*—(1) The antipernicious anemia principle found in liver, kidney, stomach and the nuclei of chicken erythrocytes² is necessary for production of a normal number of red cells of normal size. This principle has been reported to increase the red cell count and hemoglobin in normal individuals.³

(2) Iron and arsenic were reported to increase the formation of red cells and hemoglobin. They probably do not influence either the red

¹Hurtado, A. Studies at High Altitude. Blood Observations on the Indian Natives of the Peruvian Andes. *Am J Physiol* 100: 487-505 (May) 1932.

²Jones N. W. Phillips B. I. Larsell O. and Nokes H. T. The Hematopoietic Effect of Nuclear Extracts in Human Anemias. *Ann Int Med* 2: 603-621 (Jan.) 1929. More conclusive evidence of the effectiveness of chicken nuclei has been secured since this article was published (personal communication).

³Watkins C. H. Johnson R. and Berglund H. Effect of Liver Extract on Erythrocytes and Reticulocytes in Normal Individuals. *Proc Soc. Exp Biol and Med* 25: 720-21 (May) 1928.

Berglund H. Watkins C. H. and Johnson R. Statistical Significance of Erythrocyte Counts during Response to Liver Extract in Normal Individuals. *Proc Soc Exp Biol and Med* 25: 833-838 (June) 1928.

cell count or hemoglobin if given in therapeutic dosage to individuals who are not anemic

(3) Any drugs which cause rapid loss of fluid such as diaphoretics, diuretics, emetics and purgatives will cause a slight rise in the count due to concentration of the blood from diminished plasma volume

(4) Cold baths are said to cause an average increase of 1,800,000, disappearing in an hour, due to a peripheral vasoconstriction or to contraction of the spleen This may apply to capillary blood only

(5) There is often a transitory, postoperative rise of 100,000 to 1,000,000 due to dehydration and resultant decreased plasma volume

(6) Chronic carbon monoxide poisoning will produce an increase in the erythrocyte level The degree of carbon monoxide poisoning resulting from smoking¹ or exposure to the motor vehicle traffic of large cities is not sufficient to produce this effect as has been claimed

(f) *Diurnal Variations*—A little work has been done on repeated red cell counts and hemoglobin estimations at different times of day As much as 30 per cent variation in hemoglobin in one day in the same individual has been reported, but later work indicates that such large variations are unusual The cause of this variation has not been explained, but it is probably due at least in part to variations in plasma volume and the degree of contraction of the spleen

(g) *Muscular Activity*²—A slight fall in erythrocytes with a corresponding change in hemoglobin and cell volume has been reported after an hour's rest period The red cell count and cell volume are about 5 per cent lower after a period of rest in the recumbent position than during ordinary activity The difference is probably due to storage in the spleen

(h) *Menstruation*³—The average menstrual blood loss is only about 50 cc and 95 per cent of women lose less than 150 cc This amount of blood loss is not sufficient to affect significantly the hemoglobin values, although menorrhagia is one of the more common causes of anemia

C Pathologic Variations—1 **In Red Cell Counts and Hemoglobin**—(a) *Anemia*—Anemia may be defined as that condition in which the red cell count, or the hemoglobin, or both, are below the

¹ Walters O S A Comparison of Erythrocyte Count Total Hemoglobin and Corpuscular Hemoglobin in Smokers and Nonsmokers J A M A 102 1936 (June 9) 1934

² Walters O S The Erythrocyte Count Quantity of Hemoglobin and Volume of Packed Cells in Normal Human Subjects During Muscular Inactivity Am J Physiol 108 118-124 (April) 1934

³ Duckles Dorothy and Elvehjem C A Hemoglobin Studies on College Women with Special Reference to the Effect of Menstruation J Lab & Clin Med 22 607-614 (Mar) 1937

Barer Adelaide P and Fowler W M The Blood Loss During Normal Menstruation Am J Obst & Gynec 31 979-986 (June) 1936

normal limits for an individual of the patient's sex and age. It may be masked by a decrease in plasma volume as in myxedema or it may be only apparent due to an increased plasma volume as in late pregnancy. In the vast majority of cases when a relatively low red cell count and hemoglobin estimation are found, this is due to a decrease of their total quantities in the blood stream. The diagnosis of anemia should always be based on the laboratory examination, since a patient with no anemia may be pale, due to peripheral vasoconstriction as in persons who spend much time indoors or who have tuberculosis, or due to a superficial edema as in nephrosis, on the other hand a patient who has anemia may have a good color due to peripheral vasodilatation. The color of the palms of the hands is somewhat more dependable than the color of the skin or mucous membranes elsewhere. Having established the diagnosis of anemia, its type and cause must be determined. The following laboratory procedures will materially aid in this differential diagnosis and are, therefore, indicated whenever the diagnosis of anemia has been established, in addition to the routine blood examination and the securing of additional data by non laboratory procedures

- (1) Accurate color, volume, and saturation index determinations
- (2) Icterus index (and direct van den Bergh?)
- (3) Study of stained smear for the morphology of the erythrocytes and leukocytes and for malaria parasites
- (4) Gastric contents analysis, noting especially the presence or absence of achylia, and evidences of carcinoma, ulcer, or benign neoplasms
- (5) Urinalysis, noting especially the urobilinogen excretion and the presence or absence of erythrocytes, casts, albumin, or bilirubin
- (6) Stool examination, looking especially for blood and intestinal parasites or their ova
- (7) Reticulocyte count

Few cases of anemia can be considered adequately studied unless the above examinations have been performed. In many cases other studies will be indicated as well, among which are the following

- (8) Bleeding time, coagulation and clot retraction times and platelet count
- (9) Examination of moist cover slip preparations for sickle cells
- (10) Erythrocyte fragility test
- (11) Erythrocyte diameter determination
- (12) Renal function tests
- (13) Blood culture
- (14) Sternal puncture and examination of bone marrow

(15) Puncture of the spleen and lymph nodes and examination of the punctate

A discussion of the differential diagnosis of anemias will be postponed until the interpretation of these tests has been considered

(b) *Polycythemia or Erythrocytosis*—Under these terms are grouped those conditions in which the red cell count and usually also the hemoglobin estimation are above the normal limits for a person of the patient's sex and age. These may be simulated by decreased plasma volume, or masked by increased plasma volume. Hence, plasma and total blood volume determinations are indicated. The differential diagnosis will be discussed later.

2 In the Color, Volume and Saturation Indexes—Anemias are classified as macrocytic, normocytic or hypochromic microcytic by the results of the color, volume and saturation index studies as shown in Table 9. The classification of anemias in this way is of therapeutic

TABLE 9—DETERMINATION OF THE TYPE OF ANEMIA*

Color index	Volume index	Saturation index	Cell diameter in micra	Type of anemia
↑ 1.2-2.0	1.2-2.0	0.85-1.15	8.0-10.0	Macrocytic
[0.8-1.2	0.8-1.2	0.85-1.15	7.0†- 8.0	Normocytic
0.4-0.8	0.5-0.8	0.60-0.80	6.0- 7.0	Hypochromic microcytic

* Reproduced by permission of the copyright owners from Osgood E. E. and Ashworth Clanc M. Atlas of Hematology. P. 118. J. W. Stacey Inc. San Francisco 1937.

† In familial hemolytic icterus 5.5-7.0 micra.

value as well as diagnostic value since nearly all macrocytic anemias respond to adequate doses of antipernicious anemia principle and nearly all microcytic anemias to adequate doses of iron, while normocytic anemias do not respond to either liver or iron. Note that the saturation index is never high. A report of a saturation index above 1.2 indicates error in technic or calculation.

V SIGNIFICANCE OF ALTERATIONS IN THE MORPHOLOGY OF THE ERYTHROCYTES AND OF THE APPEARANCE OF IMMATURE CELLS OF THE ERYTHROCYTE SERIES IN THE BLOOD

Study Plate I and its accompanying legend, Table 42 and the illustrations in the Atlas of Hematology for details of morphology. These should be looked for especially whenever deviations from the normal in red cell count or hemoglobin are found. They may appear in the blood stream in any severe anemia except the aplastic type. The most marked variations from the normal are seen in pernicious anemia.

during exacerbations Thin, well stained smears are absolutely essential for satisfactory study of red cells

A Anisocytosis—This is a term used to designate a marked irregularity in the size of red cells It occurs in any severe anemia not of the aplastic type

1 Macrocytosis—Macrocytes are large red cells over 8 micra in diameter They are the predominating type of cell in pernicious anemia and other anemias due to a deficiency of the antipernicious anemia principle Macrocytosis may be demonstrated by measuring

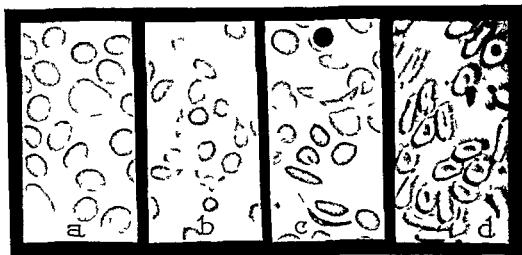


FIG 5—Types of anisocytosis and poikilocytosis All are photomicrographs with a magnification of 750 diameters a Pernicious anemia Note the preponderance of macrocytes and the apparent hyperchromia although the actual hemoglobin content of the cells is normal for their size b Hypochromic microcytic anemia of chronic blood loss Note the typical poikilocytosis the achromia and the preponderance of microcytes c True sickle cell anemia in a negro Note the characteristic crescentic cells with pointed ends and the metakaryocyte (normoblast) d Anemia of chronic infection in a white person showing elliptical cell type of poikilocytosis Note the elongated cells with rounded ends and the tailed poikilocyte near the bottom The cells in c and d average normal in size and hemoglobin content

the diameters of a large number of red cells but the volume index determination is a much simpler way to demonstrate this predominance of large cells See figure 5, a

2 Microcytosis—Microcytes are small cells less than 7 micra in diameter They may occur in the blood stream in any type of severe chronic anemia but microcytosis or a preponderance of microcytes is diagnostic of anemias due to iron deficiency, hence the low volume index and low color index in this type of anemia Microcytes are thought by some¹ to indicate fragmentation of erythrocytes and there

¹ Cooley T B and Lee Pearl The Role of Erythrocyte Fragmentation in the Genesis of Anemia J Ped 3 55 (July) 1933

Auer J The Structure and Function of Filaments Produced by Living Red Corpuscles Am J Med Sci 186 776-794 (Dec.) 1933

fore to constitute an indication of old age of the cells and of a decreased rate of red cell destruction See figure 5, b

A special type of microcytosis appears in familial hemolytic icterus where all the cells are small in diameter but the volume is normal, indicating that they are more nearly spherical than the normal red cells This anomaly of shape is diagnostic of this disease and is some times called spherocytosis They are never truly spherical however

3 Erythrocyte Diameter ¹—This determination is indicated when familial hemolytic icterus is a possibility and may be used instead of the volume index in the study of other anemias It gives a quantitative expression of the degree and character of the anisocytosis It is however, time consuming, and the results at best consider only a very few cells when compared with the billions of cells averaged in the simpler volume index determination In conjunction with the cell volume determination it aids in demonstrating the tendency of erythrocytes to assume the spheroid form in familial hemolytic icterus, but aside from this it is chiefly of research value, or to be used when enough blood for the volume index is unobtainable

(a) *Normal Values*—These differ according to different authors but the best data available indicate that the average cell diameter is about 7.6 microns with a range of 7.0 to 8.0 microns and few cells smaller than 6.0 or larger than 9.0 microns If individual measurements are made, the results are usually plotted in a curve If an eriometer or halometer is used, only the average cell diameter is determinable and even this may be impossible to determine in anemias associated with much anisocytosis

(b) *Interpretation*—An increased average cell diameter and a wider curve indicating a greater spread in cell size are characteristic of the macrocytic anemias due to deficiency of the antipernicious anemia principle A decreased cell diameter with increased range of cell diameters is characteristic of the hypochromic microcytic anemias due to iron deficiency A decreased cell diameter with a comparatively narrow range of cell size and a normal volume index is diagnostic of familial hemolytic icterus In aplastic anemia, the results are normal and in other anemias the average cell diameter is normal but there is a greater variation in cell diameters

B Poikilocytosis—This is a term used to designate a marked irregularity in the shape of red cells It occurs in any severe anemia not of the aplastic type Erythrocytes are normally almost round, but

¹ Price Jones C. Red Blood Cell Diameters. Pp 82. Oxford University Press New York and London 1933

Jørgensen S. and Warburg E. J. The Indices and Diameters of the Erythrocytes and the Best Haematological Criterion of Pernicious Anaemia. Acta Med Scandinav. 66: 109-186 and 499 1927 To this article is appended an extremely valuable and extensive bibliography

Andresen Marjory I. and Mugrage E. R. Diameter and Volume of Red Blood Cells in Infants and Small Children. Folia Haemat. 61: 201-210 1938

Haden R. L. The Volume Thickness Index of the Erythrocyte of Man. J. Lab. & Clin. Med. 20: 567-571 (Mar.) 1935

Haden R. L. Diffraction Methods for Measuring the Diameter of the Red Blood Cell. J. Lab. & Clin. Med. 23: 508-518 (Feb.) 1938

Morgensen E. Studies on the Size of the Red Blood Cells Especially in Some Anemias. Pp 216. Humphrey Milford Oxford University Press London 1938

in some diseases they may be oval, pear-shaped, or have the shape of a tennis racket, dumbbell, etc. Do not confuse this with crenation or with molding of the corpuscle due to too thick a smear. Cell shape should be judged only from erythrocytes which do not touch any other cell. See figures 5 and 6.

1 **Sickle Cells**—In *sickle cell anemia*, the red cells tend to assume a peculiar crescent shape with both ends pointed, and the proportion of these pathognomonic poikilocytes increases on standing in a moist cover slip preparation. The tendency to form "sickle" cells and also that to form elliptical cells seems to be familial but the former is limited to the negro race or persons having negro blood although a few cases in patients with no known negro ancestry have been reported. The sickle cell anomaly¹ occurs in 5 to 10 per cent of all negroes but in only a few of these does anemia occur. If a negro patient has anemia and sickling is found in moist cover slip preparations it is still necessary to prove that the anemia is not due to one of the other causes of anemia.

2 **Familial Poikilocytosis (ovalocytosis)**—Sickle cells should not be confused (see Figs. 5 and 6) with the elliptical² or sausage shaped cells characteristic of this familial anomaly of cell form. Familial poikilocytes have oval ends and are never sharp pointed as are sickle cells. The only importance of the condition is that it may be confused with sickle cell anemia. It apparently does not affect the incidence of anemia but if anemia develops from some other cause the proportion of cells of this shape may increase.

3 **Moist Cover Slip Preparations**—Such preparations should be examined whenever sickle cell anemia, malaria or filariasis is suspected. The sickle cell phenomenon should be looked for in the blood of any negro patient. It has been reported to occur in the white race⁴ but such persons almost certainly have some negro blood in their ancestry. The characteristic appearance of sickle cells in the moist cover slip preparations after standing from 12 to 24 hours is shown in Figure 6.

Malaria parasites may be observed in moist cover slip preparations as hyaline bodies containing pigment granules in rapid motion inside the red corpuscles. Filaria parasites seen in blood taken at night appear as long filaments in rapid motion which cause movement of the red corpuscles in their vicinity. They should be sought in any patient with elephantiasis or chylous ascites.

¹ Diggs L. W., Ahmann C. F. and Bibb Juanita. The Incidence and Significance of the Sickle Cell Trait. *Ann Int Med* 7: 769-778 (Dec.) 1933.

² Strauss M. B. and Daland Geneva A. Hereditary Ovalocytosis (Human Elliptical Erythrocytes). Observations on Ten Cases in One Family. *New England J Med* 217: 100-103 (July 15) 1937.

³ Hunter W. C. A Further Study of a White Family Showing Elliptical Erythrocytes. *Ann Int Med* 6: 775-781 (Dec.) 1932.

⁴ Rosenfeld S. and Pincus J. B. The Occurrence of Sicklemia in the White Race. *Am J Med Sci* 184: 674 (Nov.) 1932.

C Polychromatophilia—This is an irregularity in staining, some of the cells take a more basic stain than others and hence, appear greenish or bluish. This, of course, can not be detected if all cells are blue, due to poor technic in staining. It is due to the persistence of some of the basophilic material normally constituting the cytoplasm of the most immature cells of this series together with the presence of quantities varying from none to a full quota of hemoglobin. Some believe that polychromatophilia in nucleated cells of the erythrocyte series is an indication of the stage of maturity. It can not be a very



FIG. 6—Sickle cells as seen in a moist cover slip preparation

reliable criterion of the stage of development of the nucleated series since it is common in akaryocytes (non nucleated erythrocytes). It does indicate immaturity in the akaryocytes (non nucleated erythrocytes), however, and has exactly the same significance as basophilic stippling or reticulocytes, but the reticulocyte count is far more reliable as a criterion of the rate of erythrocyte production.

D Stippling or Punctate Basophilia—This is a condition in which there are blue staining, basophilic granules scattered through the cell. It is especially characteristic in lead poisoning, but may occur in pernicious anemia and other severe anemias. Careful search for basophilic stippling should be made whenever lead poisoning is suspected. This has the same significance as polychromatophilia or an increase in reticulocytes. Sometimes the same proportion of cells will show polychromatophilia on one portion of the slide and basophilic stippling

on another portion of the same slide. Basophilic stippling is much less common than polychromatophilia. It may be present in any of the conditions listed as causes of an increase in the reticulocyte count.

E. Achromia—This is a condition in which the centers of the majority of cells are paler than usual. It is seen in iron deficiency anemias, especially those due to chronic blood loss, but not in uncomplicated pernicious anemia. It is due to a deficiency of hemoglobin per unit volume of cell. The saturation index gives a quantitative expression of the degree of achromia present and is much more reliable because achromia may be simulated by decreased cell thickness which does not influence the accuracy of the saturation index. See figure 5, b.

F. Nucleated Red Cells—The morphology of these is given in Plate I and the accompanying legend, in Table 42 and in the illustrations in the Atlas of Hematology. The presence of nucleated erythrocytes in the blood indicates increased activity of the marrow although this increased activity may be ineffective in maintaining the blood level as in myelophthisic anemias due to quantitative deficiency in the amount of marrow available. The more marked the hyperplasia of the marrow the greater the number of nucleated red cells which may appear in the blood and the greater the proportion of the more immature forms. Nucleated erythrocytes occur normally in blood during fetal life and for a short time after birth. Nucleated red cells may occur in any severe anemia except the aplastic type but they occur in the greatest numbers during the crises of pernicious anemia. They may also occur in small pox, osteomyelitis, involvement of the bone marrow by tumors, and in the severe anemias of early childhood. Outpouring of many nucleated erythrocytes and immature leukocytes is not uncommon shortly before death or after extreme pain such as that of coronary occlusion. Karyoblasts (megaloblasts), if found, favor a diagnosis of pernicious anemia, but in many typical cases, they are difficult or impossible to find, and they may occur in other conditions, particularly lead poisoning, granulocytic (myelogenous) leukemia, erythroleukoblastosis, and the other severe anemias of early childhood. Some¹ regard karyoblasts (megaloblasts) as specific evidence of the presence of pernicious anemia.

G. Howell-Jolly Bodies and Cabot's Rings—These are small blue, red, or purplish staining dots and rings, respectively, in the red cells. They are thought to be remnants of nuclei which have been incompletely absorbed. Hence, like polychromatophilia, basophilic stippling and nucleated red cells, they are to be interpreted as immature

¹ Jones O. P. Cytological Studies of Biopsied Pernicious Anemia Bone Marrow During Relapse. Proc Soc Exper Biol & Med 34 694-696 (June) 1936

cells and, therefore, evidence of great bone marrow activity, rather than as specific for any particular type of anemia. They are especially numerous after splenectomy and are most apt to be found in anemias associated with severe changes in the spleen.

VI EVIDENCES OF THE RATE OF ERYTHROPOIESIS

A Reticulocyte Count¹—This furnishes our most reliable criterion of erythropoietic activity. The reticulocytes are increased in any condition associated with increased rate of red cell formation and decreased with decreased red cell formation. The test is, therefore, desirable in the accurate study of all anemias. It is specifically indicated in following the response of any anemia to therapy, and in any anemia in which hemolytic icterus, sickle cell or aplastic anemia are considered as possibilities, as an aid in diagnosis. In following the response of an anemia to treatment, reticulocyte counts should be repeated daily from the date of beginning treatment for 2 weeks.

1 Normal Values—Figures given by different authors vary widely. Our studies indicate that this difference in figures is due to variations in the technic used. Osgood and Wilhelm have devised a method, given on page 496, which shows the maximum number of reticulocytes. Normal values, based on a study of 110 men, 50 women and 208 children, using this method, proved to be 0.5 to 3.0 (extreme 4.0) per cent with an average of 1.5 per cent. The figures were the same for both sexes and at all ages above infancy. Since the percentage figures showed no correlation with the red cell counts, reticulocyte counts should be reported as per cent rather than as the number per c. mm. Values for the newborn are about 8 per cent the first day, 7 per cent the second day, 6 per cent the third day, 5 per cent the fourth day, 4 per cent the fifth and sixth days, and 3 per cent the remainder of the newborn period, based on a study of 200 newborn infants, about 10 of each sex being studied on each day for the first 10 days. The average for the period is 5 per cent with a range of 1 to 12 per cent including 95 per cent of results. Throughout childhood, although adult standards apply, slighter stimuli are required to produce a corresponding increase in reticulocytes.

2 Increased Reticulocytes—This indicates rapid red cell formation and hence an actively functioning marrow. The greatest increases

¹ Orten, J. M. The Properties and Significance of the Reticulocyte. *Yale J. Biol. & Med.* 6: 519-539 (May) 1934.

² Osgood, E. E., Baker, R. L. and Wilhelm, Mable M. Reticulocyte Counts in Healthy Children. *Am. J. Clin. Path.* 4: 292-296 (May) 1934.

Osgood, E. E. and Wilhelm, Mable M. Reticulocytes. *J. Lab. & Clin. Med.* 19: 120-1135 (July) 1934.

occur in familial hemolytic icterus and in sickle cell anemia. Counts of over 10 per cent (200,000 per cubic millimeter) in patients who are not receiving therapy should suggest these diagnoses and much higher counts may occur.

In untreated pernicious anemia, the reticulocytes may be normal or greatly increased during and preceding remissions. As a rule they are moderately increased in percentage, but normal or decreased in total number. After administration of the antipernicious anemia principle¹ in macrocytic anemia a typical increase begins almost immediately, reaching its maximum in 5 to 15 days after adequate therapy is started and then returning to normal. The level reached bears an inverse ratio to the red cell count when the initial red cell count is under three million. It is so definite (5 to 60 per cent) and constant in such cases that if it does not occur one is justified in concluding that the case is not one of macrocytic anemia or that the preparation used is inactive or being given in inadequate dosage. If the initial count is over 3.0 million, the increase is not so marked or constant. Table 10 shows the expected range of rise in reticulocytes.

TABLE 10—EXPECTED RETICULOCYTE PEAK FOLLOWING THERAPY*

Initial R B C millions per c mm	Average of reticulocyte peak in per cent	Range in reticulocyte peak in per cent
0.5-1.0	40.0	20.0-70.0
1.0-1.5	30.0	15.0-60.0
1.5-2.0	20.0	10.0-50.0
2.0-2.5	15.0	4.0-35.0
2.5-3.0	8.0	3.0-20.0
Above 3.0	Inconstant	1.0-15.0

* Reproduced by permission of the copyright owners from Osgood E. E. and Ashworth Clarence M. *Atlas of Hematology* J. W. Stacey Inc. San Francisco 1937 p. 117.

for each red cell level if specific therapy with liver or iron is adequate and the correct diagnosis has been made.

A similar increase in reticulocytes follows the administration of an adequate dose of iron² to patients with an iron deficiency anemia.

¹ Minot G. R., Murphy W. P. and Stetson R. P. *The Response of the Reticulocytes to Liver Therapy*. Am J Med Sc 175: 581-599 (May) 1928.

Minot G. R., Cohn, E. J., Murphy W. P., and Lawson H. A. *Treatment of Pernicious Anemia with Liver Extract: Effects Upon the Production of Immature and Mature Red Cells*. Am J Med Sc 175: 599-621 (May) 1928.

Bethell F. H. and Goldhamer S. M. *Standards for Maximum Reticulocyte Values Following Ventricular and Intravenous Liver Extract Therapy in Pernicious Anemia*. Am J Med Sc 186: 480 (Oct) 1933.

² Minot G. R. and Heath C. W. *The Response of the Reticulocytes to Iron*. Am J Med Sc 183: 110-121 (Feb) 1932.

A considerably increased reticulocyte count is the rule after acute hemorrhages, in lead or mercury poisoning, in malaria, and in leukemias, but it is not of diagnostic value in these conditions. It does indicate, however, that if the cause of the anemia can be removed, a prompt rise in the red cell count may be expected.

Reticulocytes are usually within normal limits in anemias due to chronic hemorrhage or to infection.

3 Decreased Reticulocyte Count—This indicates poor erythropoietic function and may constitute an indication for transfusion. The diagnosis of aplastic anemia either idiopathic or symptomatic should not be made if reticulocytes are not less than 0.4 per cent in the blood.

B Other Criteria—**1 Of Increased Erythropoiesis**—The presence of polychromatophilia, basophilic stippling, nucleated erythrocytes, or immature cells of the granulocyte (myeloid) series indicates hyperplasia of the marrow and are usually present in those cases with the more marked increases in reticulocyte count.

2 Of Decreased Erythropoiesis—Microcytosis and poikilocytosis are thought by some to indicate decreased erythrocyte production with compensatory decreased rate of erythrocyte destruction. This opinion is not yet definitely established. The cells above listed as increased with hyperplasia of the marrow are usually absent if there is hypoplasia.

VII EVIDENCES OF THE RATE OF ERYTHROCYTE DESTRUCTION

A The Icterus Index—This is indicated in all cases of anemia. It is increased in all conditions in which hemoglobin is being destroyed within the body, therefore, in all forms of internal hemorrhage, all forms of hemolysis, and in mechanical breakdown of cells as in malaria. It has been discussed on page 139.

B Urobilinogen Excretion in the Urine and Feces—Daily urobilinogen determinations on the urine should be run for a period of 10 days in all patients with anemia. An excretion sufficient to give a positive test in a dilution of 1 to 20 or more indicates excessive destruction of hemoglobin if disease of the liver and biliary tract can be excluded. The urobilinogen determination and icterus index are of value as a gauge of hemoglobin destruction only if disease of the liver and biliary tract can be excluded. Quantitative determinations of urobilinogen in the stools will give an approximate idea of the amount of hemoglobin destroyed. The greatest excretion may occur in hemolytic icterus.

C The Fragility of the Red Blood Corpuscles ¹—The resistance of the red cells to laking by hypotonic salt solution should be tested whenever hemolytic icterus is suspected. An increase in fragility indicates an increased susceptibility to erythrocyte destruction. It is of academic interest to determine it in any anemia, but it is rarely necessary for the diagnosis.

1. **Normal Values**—These are the same both for adults and for children. Hemolysis is first detectable in the 0.46 to 0.38 per cent sodium chloride solution and is complete in 0.36 to 0.30 per cent solution.

2. **The fragility of the red blood cells is increased in**

(a) *Familial Hemolytic Icterus (Acholuric Jaundice)*—Hemolysis may begin at 0.72 per cent and be complete at 0.45 per cent and the resistance is always definitely decreased. An acquired form of hemolytic icterus has been described but it seems probable that these are merely acute exacerbations of latent familial hemolytic icterus in patients the other involved members of whose family are not available for examination.

As far as we know at present a *decreased resistance* of the red cells to laking by hypotonic salt solution is pathognomonic of hemolytic icterus, and the diagnosis must remain in doubt until increased fragility of the red cells is demonstrated.

3. **The fragility is decreased (cells are more resistant) in the following conditions**

(a) *Other hemolytic anemias* such as lead poisoning

(b) *Pernicious Anemia*

(c) *Obstructive Jaundice* (differentiates it from hemolytic icterus)

In other anemias the fragility is usually normal but may be slightly decreased.

VIII PARASITES OF THE BLOOD

A Malaria—Malaria parasites should, of course, be looked for while studying the red cells in connection with the examination of every stained smear, but a special search for malaria parasites is indicated in patients who have repeated chills, unexplained anemia, or enlargement of the spleen. Since the parasites disappear from the blood very soon after quinine is given, therapy should not be started until the search for these parasites is completed.

Malaria is due to an infestation of the red cells by an animal parasite belonging to the class of sporozoa. It is characterized by repeated chills and intermittent fever in the acute stages with rapidly developing anemia and enlargement of the spleen which become more marked in the chronic stages. The diagnosis is established by finding the characteristic parasite in the blood smears and if this is impossible, as in some

¹ Vaughan Janet M. Red Cell Characteristics in Acholuric Jaundice. J Path & Bact 45 561-577 (Nov) 1937.

Dacie J V and Vaughan Janet M. The Fragility of the Red Blood Cells Its Measurement and Significance. J Path & Bact 46 341-356 (Mar) 1938.

Castle W B and Daland Geneva A. Susceptibility of Mammalian Erythrocytes to Hemolysis with Hypotonic Solutions. Function of Differences Between Discoidal Volume and Volume of a Sphere of Equal Surface. Arch Int Med 60 949-966 (Dec) 1937.

² For more details of the life cycles of these parasites and descriptions of their morphology, special works on tropical medicine or parasitology should be consulted.

cases of chronic or estivoautumnal malaria, in smears from sternal or splenic puncture

The detailed morphology of the different types and stages is described on page 485 and illustrated in the frontispiece and should be studied at this time

The chief diagnostic features of infestation with the tertian parasite are the progressive enlargement and paling of the red cell, the appearance of Schueffner's granules as maturity is reached, and the number of segments

Two widely different stages found in large numbers suggest a double tertian infection as the basis for daily chills

The diagnostic features of the quartan form are the finding of band forms, the smaller number of segments (6 to 12) and the decreasing size of the red cell with increasing depth of color and a brassy appearance

The estivoautumnal form is diagnosed if the characteristic ovoids or crescents are found and suspected if only ring forms which are finer than the tertian and have a greater tendency to occur in groups of two or three in one red cell can be found

Examinations should be repeated daily during treatment until they have disappeared from the blood, and then at increasing intervals of two weeks to two months for a year to make certain that there is no recurrence

Other laboratory findings in malaria which are discussed in detail elsewhere in this book are the high icterus index, increased urobilinogen in the urine, normocytic anemia, simple leukopenia, increase in rhabdocytes (staff cells), occasional leukocytosis, the presence of pigment in the neutrophils and monocytes, and the hemoglobinuria which occurs in the most severe cases and is spoken of as black water fever

B Filariasis—Examination of a moist cover slip preparation as well as stained smears for *Microfilaria bancrofti* are indicated when elephantiasis, chylous ascites or chyluria in a person from the tropics suggests this diagnosis. The *microfilaria* appear in the blood stream most commonly at night so that blood should be taken at night as well as by day in examining for this parasite. Eosinophilia and hematuria are often present but anemia is rare

C Trypanosomiasis—Moist cover slip preparations and Wright's stained smears should be searched for the presence of *Trypanosoma gambiense* in patients thought to have African sleeping sickness

D Leishmaniasis or Kala azar—Kala azar is the most important form of Leishmaniasis. It should be thought of in any person from the Mediterranean countries with enlarged spleen, normocytic anemia and leukopenia, often with relative monocytosis. The Leishman Donovan bodies diagnostic of the disease, are found in material obtained by sternal or splenic puncture

The organisms of *Leishmania infantum* may be found in children presenting similar symptoms from the same region. It is probable that this is the same disease

Oriental sore or Aleppo boil may be diagnosed if the organisms of *Leishmania tropica* which are identical in appearance with Leishman Donovan bodies are found in scrapings from the edge of a skin lesion, in a patient from South America, India or Asia Minor. Leukopenia, eosinophilia and monocytosis are common and anemia is absent.

1\ EXAMINATION OF MATERIAL OBTAINED FROM THE MARROW, SPLEEN OR LYMPH NODES

*A Examination of Bone Marrow*¹—The development of the sternal puncture technic² for aspirating bone marrow during life has made this a practical clinical procedure. The procedure gives results of great interest in most disorders of the blood or blood forming organs and is specifically indicated in cases in which a leukopenia with anemia suggests the possibility of an aleukemic phase of a leukemia or of an aplastic anemia. It has also proved of diagnostic value in multiple myeloma, Gaucher's disease, and the Hand Schuller Christian type of xanthomatosis.

Culture of human marrow³ has proved a useful research method but is not necessary for clinical diagnosis.

¹ Nordenson N. G. Studies on Bone Marrow from Sternal Puncture. Pp. 204. Bortzells I. s. s. l. t. Stockholm 1935.

² Young R. H., and Osgood L. L. Sternal Marrow Aspirated During Life. Cytology in Health and Disease. Arch. Int. Med. 55: 186-193 (Feb.) 1935.

Dameshek W., Henstell H. H., and Valentine I.leanor H. The Comparative Value and the Limitations of the Trepchine and Puncture Methods for Biopsy of the Sternal Bone Marrow. Ann. Int. Med. 11: 801-818 (Nov.) 1937.

³ Vogel, P., Frick I. A., and Rosenthal N. Hematological Observations on Bone Marrow Obtained by Sternal Puncture. Am. J. Clin. Path. 7: 436-447 (Sept.) 1937. 498-515 (Nov.) 1937.

⁴ Osgood I. F. and Muscovitz A. N. Culture of Human Bone Marrow. Preliminary Report. J. A. M. A. 106: 1888-1890 (May 30) 1936.

Osgood I. F. and Brownlee Inez F. Culture of Human Marrow. A Simple Method for Multiple Cultures. J. A. M. A. 107: 123 (July 11) 1936.

Osgood I. F. and Brownlee Inez F. Culture of Human Marrow. Details of a Simple Method. J. A. M. A. 108: 1793-1796 (May 22) 1937.

Osgood I. F. Culture of Human Marrow. Length of Life of the Neutrophils, Eosinophils and Basophils of Normal Blood as Determined by Comparative Cultures of Blood and Sternal Marrow from Healthy Persons. J. A. M. A. 109: 933-936 (Sept. 18) 1937.

Osgood I. F. The Histogenesis, Classification and Identification of the Cells of the Blood and Marrow Based on Cultures and Hematologic Studies of Human Marrow and Blood. Am. J. Clin. Path. 8: 59-74 (Jan.) 1938.

Osgood I. F. Culture of Human Marrow. Studies on the Mode of Action of Sulfanilamide. J. A. M. A. 110: 349-356 (Jan. 20) 1938.

Osgood I. F. Culture of Human Marrow. An Improved Apparatus for Large Scale Culture. Am. J. M. Sc. 105: 141-144 (Feb.) 1938.

Osgood I. F. Culture of Human Marrow. A Comparative Study of the Effects of Sulfanilamide and Antipneumococcus Serum on the Course of Experimental Pneumococcal Infections. Arch. Int. Med. 62: 181-198 (Aug.) 1938.

Osgood I. F. Culture of Human Marrow. Texas State J. Med. 34: 206-208 (July) 1938.

Osgood I. F. Culture of Human Marrow as an Aid in the Evaluation of Therapeutic Agents. Studies of Sulfanilamide and Related Compounds. J. Lab. & Clin. Med. to be published.

Osgood I. F., and Bracher C. J. Culture of Human Marrow. Studies of the Effects of Antigen Keys on Normal and Malignant Cells. Ann. Int. Med. to be published.

1 **Normal Values**—These are expressed as a percentage of the total number of nucleated cells found. The results of a study of the sternal marrow of 28 healthy males are summarized in Table 11. Similar values have been obtained in a study¹ of 24 healthy young women. In pregnant women the total nucleated cell counts were some

TABLE 11—DIFFERENTIAL CELL COUNT OF NORMAL STERNAL MARROW*

Type of cell	Average per cent	Range per cent
Neutrophil lobocytes (polymorphonuclears)	13.30	7.0-25.0
Eosinophil lobocytes	0.45	0.0-1.0
Basophil lobocytes	0.10	0.0-0.2
Neutrophil rhabdocytes (staff cells)	24.10	13.0-35.0
Eosinophil rhabdocytes	0.80	0.0-2.6
Basophil rhabdocytes	0.06	0.0-1.0
Neutrophil metagranulocytes (metamyelocytes)	7.40	1.0-10.0
Eosinophil metagranulocytes	0.64	0.0-2.0
Neutrophil granulocytes (myelocytes)	0.86	0.0-10.0
Progranulocytes S (promyelocytes I)	1.68	0.0-5.0
Progranulocytes A (promyelocytes II)	1.48	0.0-5.0
Granuloblasts (myeloblasts)	0.44	0.0-2.0
Lymphocytes	10.60	4.0-16.0
Monocytes	2.06	0.0-5.0
Metakaryocytes (normoblasts)	5.0	2.0-10.0
Karyocytes (pronormoblasts)	7.0	2.0-15.0
Prokaryocytes (erythroblasts)	1.5	0.0-5.0
Karyoblasts (megakaryoblasts)		0.0-0.2
Granulocyte erythrocyte† ratio	3.6:1.0	2.1 to 9.1

* Reproduced by permission of the copyright owners from Osgood, E. E. and Ashworth, Clarence M. *Atlas of Hematology*, p. 143. J. W. Stacey, Inc., San Francisco, 1937.

† Nucleated cells only.

¹ Pitts, H. H. and Packham, Evelyn A. *Hematology of Sternal Marrow and Venous Blood of Pregnant and Non pregnant Women*. Arch. Int. Med. to be published.

what higher but the differential counts were similar. More data are needed on the total nucleated cell count per cubic millimeter of the marrow, but preliminary studies suggest that this count is usually 6,000 to 70,000 per cubic millimeter with 95 per cent of the counts between 10,000 and 50,000.

2 **Interpretation**—Absence of, or great decrease in, nucleated cells and reticulocytes is diagnostic of aplastic anemia. A marked increase in the percentage of progranulocytes (promyelocytes) or granuloblasts (myeloblasts) is diagnostic of granulocytic (myelogenous) leukemia. In chloroma large atypical granuloblasts (myeloblasts) predominate in the marrow. The occurrence of prolymphocytes and lymphoblasts, as proved by the negative peroxidase stain, is diagnostic of acute lymphocytic leukemia or infectious mononucleosis. The percentage of lymphocytes is greatly increased in the marrow in chronic lymphocytic leukemia. Characteristic cells are found in the marrow even though absent from the blood in leukemias. In pernicious anemia there is a great increase in the percentage of karyoblasts (megaloblasts) and prokaryocytes (erythroblasts). In anemias due to iron deficiency and in anemias associated with infection, there is a great increase in the karyocytes and metakaryocytes (normoblasts). In malaria, the parasites are present in a higher percentage of red cells in the marrow than in the blood, and all stages of phagocytic digestion of the parasites by the neutrophils may be observed in the marrow, although only the pigment granules remaining from this complete digestion have been observed in the neutrophils which appear in the blood. In Leishmaniasis, the characteristic Leishman Donovan bodies will be found. In lipoid histiocytosis (Gaucher's disease, Niemann Pick's disease, and the Hand-Schüller-Christian type of xanthomatosis) the typical large vacuolated foam cells may be found. The decrease in granulocytic (myeloid) cells in agranulocytosis should differentiate it from acute leukemic leukemias with which it is so often confused. Using this method, it has been possible to prove that the karyoblast (megaloblast) is the precursor of the metakaryocyte (normoblast) and that the monoblast and promonocyte are the precursors of the mature monocyte.

B **Splenic Puncture**¹—This procedure is indicated in patients with splenomegaly in which it is not possible to establish the diagnosis by other methods. The procedure carries a slight risk, as death has been known to occur from hemorrhage following the puncture. It may be necessary to establish the diagnosis in Leishmaniasis (kala

¹ Hess, J. H. Splenic Puncture as a Diagnostic Procedure in Infancy and Childhood. *Ann. Int. Med.* 4: 467-483 (Nov.) 1930.

azar), in Gaucher's disease, in Niemann-Pick's disease, in the Hand Schuller Christian type of xanthomatosis, in some cases of chronic malaria, or in aleukemic myelosis Splenic puncture is contra indicated in any patient with prolonged bleeding or clotting time, in all hemorrhagic diseases, hemangiomas, cysts, and when malignant tumors are thought to be present

1 **Interpretation**—Normal values are not known because the procedure is contra indicated if the spleen is not enlarged In Leishmaniasis the characteristic Leishman Donovan bodies will be found in the cells when stained with Wright's stain In the three types of histiocytosis (Gaucher's disease, Niemann Pick's disease, and the Hand Schuller Christian type of xanthomatosis) the characteristic foam cells will be found in smears These are monocytes which have phagocytized so much lipoid material that they are greatly increased in size and have a foamy striated appearance In cases in which chronic malaria is suspected, but the presence of the organisms cannot be demonstrated in the blood even after the administration of adrenalin, they may be demonstrated in the material obtained from splenic puncture In aleukemic myelosis, numerous progranulocytes (promyelocytes) and granuloblasts (myeloblasts) will be found

C Lymph Node Puncture—Lymph node puncture is indicated in patients with generalized enlargement of the lymph nodes in whom a diagnosis has not been established by other methods

Local anesthesia and a sternal puncture needle may be used but in many cases it is difficult to obtain enough material by puncture for satisfactory preparations The puncture material is smeared on slides and stained with Wright's stain The normals are not yet known Presumably normal smears would show many lymphocytes with a few prolymphocytes and lymphoblasts In Hodgkin's disease, giant cells and eosinophils may be seen In lymphosarcoma or acute lymphocytic leukemia, many lymphoblasts would be seen More experience with the method is needed before its clinical value is known Removal and section of a lymph node, making fresh imprint preparations as well as paraffin sections will usually be necessary in addition before the proper interpretation of puncture material is learned

X DIFFERENTIAL DIAGNOSIS OF ANEMIAS¹

It was formerly customary to divide anemias into two groups Under the term primary anemia were included cases of pernicious

¹ Weil P E, Isch Wall P, and Perles Suzanne Diagnostic de la Maladie de Hodgkin par la Ponction ganglionnaire Presse méd 44 1540-1543 (Oct 3) 1936

Kirschbaum A and Downey H A Comparison of Some of the Methods Used in Studies of Hemopoietic Tissues Anat Rec 68 227-234 (May) 1937

² Witts L J Goulstonian Lectures on the Pathology and Treatment of Anemia Lancet Pp 495, 549 601 and 653 (March 5 12 19 and 26) 1932

Osgood F E and Haskins H D Causes Classification and Differential Diagnosis

anemia and sometimes chlorosis, and under the name secondary anemia, all others. The bases for these names were that the cause was supposed to be unknown in primary anemias and known in secondary anemias. As this classification is illogical in grouping unrelated conditions together and tends to discourage thinking, it is now obsolete. Instead, the classification into macrocytic, normocytic and hypochromic microcytic anemias (Table 9) which aids materially in diagnosis and planning therapy is used. Since clinical anemias are often due to the combined presence of several fundamental causes anemias are more easily understood if the three fundamental causes of anemia in their pure forms are first described.

A Fundamental Causes—Obviously there are only three ultimate causes of anemia.

1 *Deficient production* of red cells, or of hemoglobin, or of both, either due to lack of materials or to lack of active blood-forming tissue.

2 *Abnormally rapid destruction* of red cells or hemoglobin, or both, in the body are included in this group.

3 *Hemorrhage* or loss of red cells and hemoglobin from the body.

As all clinical anemias result from various combinations of these factors they deserve special study.

1 **Deficient Production of Red Cells**—This is the group about which we know least.

(a) *Deficient Erythrocyte Building Material*—Iron, certain other metals, and probably other as yet unidentified substances are necessary for the formation of hemoglobin. The antipernicious anemia principle is necessary for the formation of red cells of normal size and hemoglobin content.

The iron deficiency anemias include nutritional anemia, the anemia of chronic hemorrhage, idiopathic hypochromic anemia, and chlorosis. In nutritional anemia, the iron deficiency is due to an inadequate intake, in chronic hemorrhage, to an excessive loss of iron, in hypochromic anemia, to deficient absorption of iron because of inadequate digestion of organic iron compounds, and in chlorosis probably to a combination of these factors. These anemias are characterized by low color, volume and saturation indexes and are called hypochromic microcytic anemias.

Anemias due to deficiency of the antipernicious anemia principle or of the intrinsic or extrinsic factors necessary for its production include pernicious anemia, pernicious anemia of pregnancy, and the anemias of sprue and *Diphyllobothrium latum* infestation. In pernicious anemia the deficiency is in the intrinsic factor, in pernicious anemia of pregnancy, the deficiency is due to an inadequate supply of the antipernicious anemia principle for the needs of both

of Anemias. Based on the Detailed Examination of Over Two Hundred Patients and a Study of the Literature. *Ann Int Med* 5: 216-236 (May) 1912.

Also in L. J. and Ashworth: *Clarence M. Atlas of Hematology*. 1p. 255. J. W. Stacey, Inc. San Francisco 1933.

Castle W. B. and Minor C. K. *Pathological Physiology and Clinical Description of the Anemias*. In Christian H. A. *Oxford Medicine*. New York: Oxford University Press 1936 vol. 2 part 3 pp. 539-650.

mother and fetus, and in sprue and *Diphyllobothrium latum* infestation, to deficient absorption. These anemias are characterized by high color and volume indexes and a normal saturation index and are sometimes called macrocytic anemias.

The other anemias, except those due to deficiency of iron or antipernicious anemia principle, are normocytic with the exception of the anemia of chronic blood loss which is actually a relative iron deficiency and is hypochromic microcytic as are other iron deficiency anemias.

Vitamin C,¹ vitamin B₁₂,² vitamin B₆,³ vitamin K⁴ and thyroid secretion have also been shown to be necessary for proper red cell maturation.

(b) *Aplasia of Erythropoietic Tissue*—Here we expect absence of evidences of red cell regeneration. Therefore, reticulocytes, polychromatophilic cells, and nucleated red cells should be absent from the blood stream, and, as the other marrow cells are seldom⁵ spared, one would expect, also, leukopenia affecting chiefly the granulocyte (myeloid) series and thrombopenia with the associated prolonged bleeding time, delayed clot retraction and hemorrhagic tendency. Certain poisons, especially benzol and drugs containing the benzol ring⁶ are known to produce this syndrome, and it is probable that also some bacterial toxins can produce it. It may be produced by excessive exposure of blood forming tissue to roentgen-rays or radio-active substances⁷ and in some cases is due to almost complete destruction of marrow by invasion of other tissue as in osteosclerotic anemia, and in rare instances of amyloidosis and extensive myelophthisic anemias. A few cases occur for which the cause has not been determined and they are grouped under the term idiopathic aplastic anemia. The only therapy that can be expected to be effective in the aplastic cases is

¹ Mettler S R. and Chew, W B. The Anemia of Scurvy. Effect of Vitamin C Diet on Blood Formation in Experimental Scurvy of Guinea Pigs. *J Exper Med* 55 971-979 (June) 1932.

² Gyorgy P. Robschtein Robbins F S, and Whipple G H. Lactoflavin (Riboflavin) Increases Hemoglobin Production in the Anemic Dog. *Am J Physiol* 122 154-159 (Apr) 1938.

³ Fouts P J. Helmer O M, Lepkovsky S and Jukes T H. Production of Microcytic Hypochromic Anemia in Puppies on Synthetic Diet Deficient in Rat Antidermatitis Factor (Vitamin B₆). *J Nutrition* 16 197-207 (Aug 10) 1938.

⁴ Thayer S A. McKee R W, MacCorquodale D W and Doisy E A. Recovery from the Anemia Caused by a Diet Deficient in Vitamin K. *Proc Soc Exper Biol & Med* 37 417-420 (Nov) 1937.

⁵ Baar, H. Progressive postinfektiöse Erythrophthise. *Folia haemat* 35 111-115 (November) 1927. This extremely rare condition in which only erythropoiesis is disturbed is the third member of the group of specific bone marrow dysfunctions: agranulocytosis and some types of thrombopenic purpura are the other two. In aplastic anemia all three functions are impaired.

It seems probable that these four conditions may be merely different responses of different individuals to different quantities of the same toxins. For example it is possible experimentally to produce pictures closely simulating any one of these syndromes by varying the doses and the duration of exposure to benzol. A further point suggesting that this is true is that cases occur showing clinical features intermediate between any two of these conditions.

It is a striking fact that all the conditions associated with a marked deficiency in mature neutrophils in the circulating blood (agranulocytic angina, aplastic anemias and acute leukemias) are clinically characterized by ulcerative or gangrenous lesions in the mouth and throat. It is now established that such lesions are the result rather than the cause of the extreme neutropenia.

⁶ Selling, L. and Osgood E E. Action of Benzol Roentgen Rays and Radioactive Substances on the Blood and Blood Forming Tissues in Downey H. *Handbook of Hematology*. Paul B Hoeber Inc. New York 1938 vol IV pp 2693-2801.

removal of the cause and restoration of bone marrow function, although transfusion may cause temporary benefit. It is theoretically possible that this deficient function might be due to absence of a normal stimulus.

The only compensatory mechanism available to the body to combat decreased formation of red cells and hemoglobin is to prolong the life of those formed (decreased rate of destruction). The evidences of this are decrease in the icterus index below 2.5, decrease in urobilinogen in the stools, and a greater tendency for it to be absent from the urine. It is possible, but by no means certain, that poikilocytosis and microcytosis are evidences of abnormal length of life in erythrocytes. As in aplastic anemia the same changes affect the neutrophils, it is to be expected that they also will show evidence of decreased rate of destruction (increased proportion of segmented forms with five or more nuclear subdivisions).

(c) *Destruction of Bone Marrow* (myelophthisic anemia) —This is usually due to invasion by other tissue as in leukemias, myeloma, malignant tumors involving the marrow, osteosclerosis, and amyloidosis but may be due to extensive osteomyelitis. Although there is often an absolute deficiency in marrow, there is a tendency for that near the lesion to be irritated to abnormal activity as evidenced by unusually immature red and white cells in the blood stream and for uninvolved marrow to be capable of compensatory hyperactivity. Attempts at compensation by decreased blood destruction may also occur. Hence, the characteristic findings are those of the causative disease plus immature and old cells of the erythrocyte series and immature and old cells of the granulocyte series, thus giving rise to a very bizarre blood picture. The color, volume and saturation indexes are variable but most often within normal limits. A low icterus index and decreased urobilinogen excretion are present when decreased red cell destruction occurs. Removal of the cause is the only therapy likely to be of benefit.

2 *Increased Rate of Red Cell Destruction within the Body* —This may be due to extravasation of blood, to hemolysis, to destruction of red cells in the blood stream as in malaria, to hyperactivity of the normal blood cell destroying mechanisms, to the production by the bone marrow of red cells with decreased resistance to the factors normally tending to destroy them, or to some abnormality in another organ (spleen?) affecting the red cells in such a way as to decrease their resistance.

The evidences of increased rate of red cell destruction will be common to all.

If no other factor than increased blood destruction plays a part, there will be evidence of rapid regeneration of cells, because the remnants of the destroyed corpuscles and hemoglobin within the body insure the continuous presence of an excess of erythrocyte and hemoglobin-forming materials.¹ Hence, one would expect to find in a purely internal blood destruction anemia, an increase in immature cells of the erythrocyte series and other evidences of increased bone marrow activity such as simple leukocytosis with increased proportion of immature forms and thrombocytosis.

Rapid regeneration of cells by the bone marrow is the compensatory mechanism in this group, and in uncomplicated cases, removal of the cause is the only

¹ It is theoretically possible that interference with the transport mechanism would prevent these from being available at the point where they are needed.

therapeutic measure indicated, for the body already contains an excess supply of blood-forming materials, from the destroyed cells

It is further obvious that if regeneration keeps pace with cell destruction, a cause for anemia of this type can exist without the production of an actual anemia, but the evidence of rapid erythrocyte destruction and of rapid erythrocyte regeneration will, nevertheless, be present

3 **Blood Loss from the Body**—This includes all types of external hemorrhage, as well as hemorrhages from the air passages and gastrointestinal tract, in which the blood leaves the body before destruction and reabsorption. Here, evidences of blood destruction will be lacking, but as long as adequate supplies of blood-forming materials are available, evidences of rapid red cell regeneration will be present. Therefore, the pictures for acute blood loss and chronic blood loss will be different

(a) *Acute Blood Loss*—The deficiency in red cells and hemoglobin will not be apparent until increased plasma volume occurs. Compensation is by rapid regeneration from existing stores, so the evidences of increased bone marrow activity including reticulocytosis, polychromatophilia, nucleated reds, simple leukocytosis, and thrombocytosis dominate the picture. Cells of normal size and hemoglobin content giving normal color, volume, and saturation indexes are formed as long as the supplies of stroma- and hemoglobin-building materials are not exhausted. Later, decreased color, volume, and saturation indexes may occur if the loss of blood was extreme, and decreased blood destruction with low icterus index, and poikilocytosis may occur as a compensatory factor, but these changes are never present in the first few days unless they were present before the hemorrhage

(b) *Chronic Blood Loss*—Here, the exhaustion of the hemoglobin-building (and probably also of stroma-building) material becomes the dominant factor, and this relative insufficiency gives rise to a picture identical with absolute iron deficiency. Thus, evidences of compensatory decreased blood destruction and, to a less extent, of compensatory increased blood formation are both present, but the most characteristic change is a decrease in the color, volume, and saturation indexes, particularly the latter. Removal of the cause and administration of iron are obviously both indicated. Here, too, it is possible for regeneration to keep pace with loss and, when the total blood volume and the normal rate of regeneration are considered, it is evident that the total quantity of blood lost per day must be very considerable (actual amount unknown) to produce anemia, if no additional factors are present

B Clinical Classification of Anemias¹—Unfortunately, clinical anemias are usually due to a combination of the above mentioned fundamental causes, and are in many instances too inadequately studied

¹ Haden R L. Clinical Significance of Volume and Hemoglobin Content of the Red Blood Cell. *Arch Int Med* 49 1032-1037 (June) 1932

Osgood E E, Haskins H D, and Trotman F E. The Value of Accurately Determined Color Volume and Saturation Indexes in Anemias Based on a Study of over 200 Patients. *J Lab and Clin Med* 17 859-886 (June) 1932

Ottenberg R. Reclassification of the Anemias. *J A M A* 100 1303-1311 (April 29) 1933

Wintrobe M M. Anemia Classification and Treatment on the Basis of Differences in the Average Volume and Hemoglobin Content of the Red Corpuscles. *Arch Int Med* 54 256-280 (Aug) 1934

for one to be certain which of these factors plays the major role. Where evidence is available, the fundamental factors present are suggested. The attempt has been made to group them in such a way as will most greatly facilitate clinical diagnosis and treatment. After anemia has been proved to be present by a red cell count and hemoglobin estimation, the type of anemia should be determined by color, volume and saturation index determinations. The fundamental cause and associated condition should then be diagnosed. The diagnosis of any anemia should consist of the type of anemia, the fundamental cause and the associated condition (for example, hypochromic microcytic anemia of iron deficiency due to chronic hemorrhage from carcinoma of the stomach, or normocytic aplastic anemia of chronic benzol poisoning).

The points of most differential diagnostic value have been indicated by italics.

C Macrocytic Anemias—The blood findings are similar in all macrocytic anemias to those described for pernicious anemia so only the points in the differential diagnosis from pernicious anemia will be given for the others. Pernicious anemia is much the most common of the macrocytic anemias. The presence of macrocytic anemia constitutes a specific indication for intramuscular administration of liver extract in adequate doses to produce a satisfactory rise in reticulocytes, in red cell count, and to keep the red cell count above 50 million.

1 Pernicious Anemia—This is a disease of middle and later life characterized by pallor, weakness, sore tongue, gastrointestinal, neurologic and mental symptoms, and a tendency to remissions. No proved case has so far been permanently cured, but adequate intake of the antipernicious anemia principle will cause all the blood findings to revert to normal, with a *corresponding improvement in the general condition of the patient*. If the special therapy is withdrawn, however, a relapse occurs. The neurologic disturbances usually improve only slightly. The diagnostic points listed below apply only to cases which have not had this treatment, as all findings, with the exception of the achlorhydria, return to normal after adequate treatment.

The volume and color indexes are high ranging usually between 1.2 and 2.0.

The saturation index is normal. This shows that the high color index is due to the increase in average volume of the red cell and not to an increase in the concentration of hemoglobin within the red cell.

The icterus index ranges from 6 to 20. It is highest during periods of rapid blood destruction. The direct van den Bergh reaction is negative. Urobilinogen is usually increased in the urine and stools.

pernicious anemia principle after expulsion of the worm. The diagnosis of macrocytic anemia due to liver disease is based on the failure of response to antipernicious anemia principle and the presence of the physical signs and laboratory tests described which are characteristic of impaired liver function. The marrow findings described under the heading of myelophthasic anemias will differentiate the anemias due to multiple myeloma and leukemia from pernicious anemia. The macrocytic anemias of childhood offer no difficulty because pernicious anemia does not occur in children. These diseases are discussed under anemia of childhood.

3 Differential Diagnosis of Pernicious Anemia from Normocytic and Hypochromic Microcytic Anemias with Which It May Be Confused—In practice, other anemias are more often mistaken for pernicious anemia, although the blood findings if studied by accurate methods readily serve to make the differential diagnosis. These are the anemias associated with carcinoma of the stomach, subacute bacterial endocarditis, lead poisoning, myxedema, chronic malaria, or familial acholuric jaundice. These all give color and volume indexes within the range of normal or lower if they are accurately determined and show other criteria of differential value which are listed under the discussion of these anemias. They are often very difficult to distinguish from pernicious anemia by non laboratory methods.

D Hypochromic Microcytic Anemias¹—These anemias could well be called hypoferric anemias as suggested by Alt. In all of them, the blood picture is essentially the same. The most characteristic feature is the low color, volume and saturation indexes with a corresponding small cell diameter and hypochromia observable in the stained smears. The red cell count is often relatively high and may be normal. It tends to be lower in the cases due to hemorrhage than in the other types of hypochromic microcytic anemias. A few nucleated erythrocytes and a slight increase in reticulocytes are not infrequently present. Poikilocytosis, anisocytosis, and polychromatophulia are usually present. The white cell count and the platelet count are normal or slightly decreased. There is no evidence of increased blood destruction. The

¹ Bethell F H, Goldhamer S M, Isaacs R and Sturgis C C. The Diagnosis and Treatment of the Iron Deficiency Anemias. J A M A 103 797-802 (Sept 15) 1934.

Davidson L S P, and Leitch I. Nutritional Anaemias of Man and Animals. Nutrition Abstr & Rev 3 901-930 (Apr) 1934.

Fowler W M and Barer Adelaide P. The Etiology and Treatment of Idiopathic Hypochromic Anemia. Am J M Sc 194 625-635 (Nov) 1937.

Fowler W M and Barer Adelaide P. Iron Deficiency Anemias. J A M A 112 110-112 (Jan 14) 1939.

Heath C W, and Patek A J Jr. The Anemia of Iron Deficiency. Medicine 16 267-350 (Sept) 1937.

icterus index and urobilinogen excretion in the urine are either normal or decreased. The sternal marrow shows an increase in metakaryocytes (normoblasts) and karyocytes (pronormoblasts) but no increase in karyoblasts (megaloblasts). All anemias of this type respond with an increase in reticulocytes and a return to normal after adequate doses of iron (1 to 3 grams of ferrous sulphate or 4 to 6 grams of ferric ammonium citrate per day). A low saturation index is a specific indication for administration of iron.

1 **Chronic Hemorrhage**—This is the commonest cause of hypochromic microcytic anemia. Bleeding hemorrhoids and the various types of menorrhagia and metrorrhagia are the commoner causes of this picture in uncomplicated form. A *low saturation index* should always lead one to search for chronic blood loss as the cause of an anemia, whether the source of the bleeding is immediately evident or not since it is obvious that the bleeding may have stopped when the patient is first seen. Conversely, a saturation index above 0.95 would be a point against chronic blood loss being the primary cause of the anemia, even when bleeding is present. Hypochromic microcytic anemia is a common complication of malignant tumors or ulcerations of the gastrointestinal tract, even though the picture may be clouded by other causes of anemia (infection, toxemia, bone marrow metastases, etc). In the hemorrhagic diseases, an anemia of acute or chronic blood loss may be added to their typical syndromes. Hypochromic microcytic anemia may result from any of the conditions listed as causing gross hematuria, gross blood in the feces or gross hemoptysis.

Carcinoma of the stomach and of the cecum are especially apt to have severe anemia as a dominant symptom, and may resemble pernicious anemia in clinical findings, in the achlorhydria and in the presence of many nucleated red cells. The volume index easily differentiates them, however. Hypochromic microcytic anemia with blood in the stool in middle aged persons without symptoms constitutes an indication for thorough fluoroscopic examination of the cardia of the stomach and the cecum, since bleeding carcinoma in these areas may be present without producing other signs.

2 **"Idiopathic" Hypochromic Microcytic Anemia**¹—This condition is sometimes called simple achlorhydric anemia which is a better term but not as frequently used. It occurs most commonly in women of middle age and is characterized clinically by the gradual onset of pallor, weakness, sore tongue, gastrointestinal disturbances, and often

¹ Wintrobe M. M. and Beebe R. T. Idiopathic Hypochromic Anemia. *Medicine* 12: 187-243 (May) 1933.

menorrhagia Physical examination reveals atrophic glossitis, enlargement of the spleen, and spoonlike depressions of the finger nails Achlorhydria or hypochlorhydria occurs in most of the cases The term "idiopathic" is a misnomer because it has been shown¹ that the cause is deficient absorption of iron because of poor digestion of organic iron compounds Inadequate diets and bleeding are frequent contributory factors

3 Chlorosis² or green sickness —This is a disease of adolescent girls, formerly common, but now very rare Recovery is rapid after the administration of iron

4 Nutritional Anemias³—The commoner types are infants kept too long on an exclusive milk diet (deficient iron intake), the anemia of premature infants⁴ (exhaustion of inadequate iron store in the liver before the usual time of change to non milk diet), and the anemias that develop in persons on very restricted diets (esophageal stenosis food fads etc) The anemias of diaphragmatic hernia and thoracic stomach are probably due to a combination of dietary restriction poor digestion and absorption, and blood loss The common hypochromic microcytic anemia of pregnancy is due to a relatively deficient iron intake for the needs of both mother and fetus

E Normocytic Anemias —1 Anemias Due to Infection —A large proportion of clinical anemias fall in this group They are often grouped under the term hemolytic anemias, but it is very doubtful whether hemolysis plays the major role in most of them Relatively few can be diagnosed by laboratory measures, alone Depression of bone marrow function and blood destruction within the body occur in most cases in varying proportions The former usually predominates, but more study with the object of determining these factors is indicated

The anemia is normocytic The blood picture suggests decreased blood formation and increased destruction in varying degrees Reticulocytes are usually normal or low, as is the icterus index⁵ The white cell count is that characteristic of the cause of the anemia Only diagnostically significant variations from the above picture will be mentioned Infections with the hemolytic organisms or idiosyncrasy to sulfanilamide may give normocytic anemia of the internal blood destruction type Infections complicated by chronic hemorrhage or inadequate iron intake may give a hypochromic microcytic anemia

¹ Mettler S R Kellogg F and Rinehart J F Chronic Idiopathic Hypochromic Anemia Am J Med Sci 186 694-704 (Nov) 1933

² Patek A J, Jr, and Heath C W Chlorosis J A M A 106 1463-1466 (Apr 25) 1936

³ Minot G R The Anemias of Nutritional Deficiency Etiology Diagnosis, Treatment and Prevention J A M A 105 1176-1179 (Oct 12) 1935

⁴ Merritt Katherine K and Davidson L T The Blood During the First Year of Life II The Anemia of Prematurity Am J Dis Child 47 261-301 (Feb) 1934

⁵ Douglas A H and Tannenbaum H The Mechanism of Secondary Anemia Arch Int Med 45 248-256 (Feb) 1930

The important subdivisions follow

(a) *Acute and Subacute Infections*—In the majority of acute infectious diseases, a moderate anemia develops late. It is most marked in smallpox (myelophthasic blood picture), in acute rheumatic fever, and in the diseases in which a hemorrhagic factor is added (typhoid dysentery, and ulcerative colitis). Bacteremias of all types, especially streptococcus bacteremias, produce a severe anemia in which the internal blood destruction picture may predominate. In prolonged fever associated with normocytic anemia for which no cause is apparent, subacute bacterial endocarditis, other alpha hemolytic streptococcus (viridans) infections or Hodgkin's disease often prove to be the explanation.

Focal infections in the sinuses or gall bladder are particularly apt to be associated with anemia. The hemolytic picture predominates in some of these infections.

(b) *Chronic Infections*—Chronic focal infection or accumulations of pus (abscesses, empyema, etc.) are extremely common causes of anemia. Syphilis and tuberculosis may cause extreme anemia or, more often none at all. Anemia is seldom marked in tuberculosis¹ not complicated by secondary infection or hemorrhage, but whether the secondary infection, the tuberculosis itself or absorbed products of tissue destruction is the chief etiologic factor in the anemias which do occur is disputed.

(c) *Acute and Subacute Glomerular and Chronic Diffuse Nephritis*²—This group is mentioned separately to call attention to the diagnostic and prognostic value of the anemia (decreased marrow function type³) which is constantly present (absent in nephrosis and most cases of hypertensive cardiovascular renal disease) and tends to increase and decrease with the severity of the renal impairment. The hematuria is shown not to be a factor in the anemia by the normal indexes and the small amount of the daily blood loss.

In normocytic anemias due to infection removal of the cause and blood transfusion are the only therapy of value. If chronic hemorrhage or dietary deficiency is associated and the anemia is of the hypochromic microcytic type iron will be of value.

¹ Braverman M. M. The Anaemia of Pulmonary Tuberculosis. *Am Rev Tuberc* 38: 466-490 (Oct.) 1938.

² Brown G. E. and Roth G. M. Anemia of Chronic Nephritis. *Arch Int Med* 30: 817-840 (Dec.) 1922.

Nordenson N. G. The Bone Marrow in the Anemia of Chronic Nephritis. *Folia Haemat* 59: 1-16 1938.

³ Notwithstanding the evidences of hypofunction of the marrow seen in the blood the sternal marrow shows an excess of nucleated erythrocytes.

2 **Internal Blood Destruction** — (a) *Internal Hemorrhage* — The physical findings in the acute cases are those of shock plus localizing signs from the site of the hemorrhage. In the early stages the diagnosis must be established by clinical findings. The icterus index will be increased within 4 to 24 hours and this sometimes occurs in hemorrhages high in the gastrointestinal tract. Do not judge the extent of the hemorrhage by the red cell count or hemoglobin until 12 to 48 hours after it has ceased. The commonest causes of acute internal hemorrhage large enough to result in anemia are peptic ulcer, ruptured ectopic pregnancy, varicosities in the esophagus or stomach (portal obstruction), and results of operation or trauma (wounds, ruptured viscera, etc). Laboratory tests are of chief value in noting the progress of recovery. Other causes of internal hemorrhage which may give rise to an increased icterus index and increased urobilinogen excretion but rarely cause anemia are intracranial hemorrhage, hemorrhagic infarction, and dissecting aneurysm.

(b) *Parasitic Diseases* — Malaria, Leishmaniasis and infestation with the intestinal parasites are the most important members of this group. In these, the excessive internal blood destruction picture dominates and bone marrow function is less depressed, although leukopenia is common. Anemia is seldom absent in malaria, but is present in only a fraction of patients infested with intestinal parasites. In hook worm infestations and amebic dysentery, hypochromic microcytic anemia of blood loss occurs. The regenerative evidence in the blood may cause these anemias to be mistaken for pernicious anemia, if accurate volume indexes are not determined. The diagnosis is established by finding the parasites and noting the course of the anemia after eradication of the cause. The treatment is specific therapy for the particular parasite with blood transfusions if the anemia is severe, and administration of iron only if hypochromic microcytic anemia is present.

(c) *Poisons* Lead, mercury, sulfanilamide,¹ phenylhydrazine snake venom, and many other poisons are included in this group. In lead² and mercury poisoning, basophilic stippling is usually demonstrable. Internal destruction of red cells with active marrow regeneration is evident from the tendency to increased icterus index and urobilinogenuria, the high reticulocyte counts, and often enormous numbers of nucleated red cells, including karyoblasts (megaloblasts), in the blood stream. The red cell count is seldom below 3 million.

¹ Wood, W. B. Anemia during Sulfanilamide Therapy. J. A. M. A. 111: 1916-1919 (Nov. 19) 1938.

² Aub, J. C., Fairhall, L. T., Minot, A. S. and Reznikoff, P. Lead Poisoning. Medicine 4: 1-250 (Feb. May) 1925.

Phenylhydrazine poisoning gives an extreme internal blood destruction picture, with high icterus index and urobilinogenuria, with no depressant and apparently even a stimulating effect on the marrow. Simple leukocytosis is the rule and reticulocytes, nucleated red cells, and immature leukocytes are usually increased in the blood. It is of importance because used in the treatment of polycythemia vera.

Certain snake venoms result in extreme hemolysis with hemoglobinuria and no interference with regeneration if the patient survives.

The treatment is removal from exposure to the responsible poison and specific therapy for the particular type of poisoning. Transfusions may be necessary in the more severe cases. Iron and liver are of no value.

(d) *Hemolytic Icterus* (acholuric jaundice) —A familial and an acquired form are described. Both are rare. It is probable that the acquired form is only a less marked degree of the same disturbance as in the familial form.

Familial hemolytic icterus is characterized by the early onset (before 10, usually very soon after birth) of slight to marked icterus, enlargement of the spleen, and moderate anemia.

The color, volume and saturation indexes are normal. The *icterus index* is high (10 to 100). The direct van den Bergh test is negative and bilirubin is absent from the urine unless obstructive jaundice is superimposed from a gallstone in the common duct as not infrequently occurs. *Urobilinogen* is greatly increased in the urine and stools except during a gallstone obstruction.

Neutrophilia with thrombocytosis and evidence of extremely rapid red cell regeneration dominate the blood picture. Reticulocytes are usually over 10 per cent, some times over 40 per cent. Polychromatophilia and nucleated red cells are numerous. Karyoblasts (megaloblasts) are sometimes found. Notwithstanding the normal volume index, microcytosis is evident in the stained smear showing that the cells tend toward the spherical form.

The most characteristic feature, without which the diagnosis may be in doubt, is a decreased resistance of the red cells to laking by hypotonic salt solution as demonstrated by the fragility test. This test will differentiate it from all other conditions. Notwithstanding these changes the patients seem to feel comparatively well. There is a definite tendency to remissions and exacerbations. They often live well into adult life without treatment.

The acquired form differs only in a less obvious familial incidence, a tendency to become manifest first in adult life, a greater contrast between the remissions and exacerbations, and the fact that often only red cells which have been washed free from plasma by repeated centrifugation after suspension in normal saline show the typical increased fragility. This is less marked during remissions and at times absent entirely.

The cause of the anemia in hemolytic icterus seems to be purely excessive blood destruction with no impairment of the erythropoietic system. Whether the excessive destruction is due to the action of a normal or abnormal reticuloendothelial system or abnormally active or both

is uncertain¹ The benefit of splenectomy is compatible with either hypothesis It is noteworthy that the increased fragility and spherical cell form persist after removal of the spleen Splenectomy during a remission produces clinical cure but is not necessary in the mildest cases If one patient with the disease is found other members of the family should be examined

(e) *Sickle Cell Anemia*²—Sicklemia is an abnormality of the red cells which appears to be inherited as a Mendelian dominant and with rare exceptions, is confined to the negro race It has been described on page 191 and is present in 5 to 10 per cent of all negroes but normocytic anemia is associated in only a small percentage of those with the sickling phenomenon

The icterus index is increased (6 to 30) as is urobilinogen excretion

The red cell count and hemoglobin are usually moderately decreased, but counts below 1.5 million are reported

The white blood cells are usually increased (10,000 to 20,000)

The characteristic feature is the tendency for *the red cells to assume a crescentic shape with both ends pointed* This occurs in dry smears but is most marked in moist coverslip preparations which have been allowed to stand a few hours (Fig 6) Evidences of both increased erythropoiesis and red cell destruction are present Nucleated red cells are often found The reason for the increased rate of red cell destruction is unknown³ It is possible that these peculiarly shaped cells are less resistant to destruction within the body although they are not less resistant to destruction by hypotonic saline in the fragility test

It must be differentiated from the tendency to formation of elliptical or oval cells which may occur in either the white or black race, and does not constitute, alone, a cause for anemia (Fig 5)

(f) *Hemoglobinuria and Hemoglobinemia*⁴—In all the conditions listed on page 22 as causes of hemoglobinuria, anemia of the internal blood destruction type may occur In the milder cases anemia may occur without gross hemoglobinuria and the diagnosis is established by identification of hemoglobin in serum obtained with special precautions to prevent hemolysis

¹ See footnote p 99

² Hahn E V Sickle Cell (Drepanocytic) Anemia Am J Med Sc, 175 206-217 (Feb) 1928

Huck J G Sickle Cell Anemia Bull Johns Hopkins Hosp 34 335-344 (Oct) 1923

Diggs L W The Erythrocyte in Sickle Cell Anemia J A M A 112 695-700 (Feb 25) 1939

³ See footnote p 209

⁴ Hamburger L P and Bernstein A Chronic Hemolytic Anemia with Paroxysmal Nocturnal Hemoglobinuria Am J M Sc 192 301-316 (Sept) 1936

Scott R B Robb Smith A H T, and Scowen E F The Marchiafava Michel Syndrome of Nocturnal Haemoglobinuria with Hemolytic Anemia Quart J Med 7 95-114 (Jan) 1938

(g) *Lederer's Acute Febrile Hemolytic Anemia*¹—This is characterized by sudden onset of fever and rapidly progressive anemia often with hemoglobinemia and hemoglobinuria. The etiology is unknown. Recovery occurs if a sufficient number of blood transfusions can be given to maintain life over a period of a few weeks.

The treatment for the other anemias associated with hemoglobinuria is prevention of exposure to cold, treatment of syphilis in paroxysmal hemoglobinuria, avoidance of the allergin in favism, and removal from the causative agent and transfusions if the anemia is severe in the others.

3 *Myelophthisic Anemias*—The characteristic laboratory findings for this group have been described (p. 203). The diagnosis is based on the bizarre blood picture and clinical evidence of bone marrow involvement. The diseases in which this blood picture predominates are leukemias and multiple myeloma. It may predominate in osteomyelitis and in metastatic tumors of bones including sarcoma, carcinoma, hypernephroma, lymphosarcoma and Hodgkin's disease. The presence of this blood picture should always cause one to think of bone marrow involvement and except in leukemias in which the diagnosis is obvious from the blood study, is an indication for examination of the sternal marrow and for roentgenographic study of the bones. Osteosclerosis² is a rare disease which sometimes gives this picture in the early stages and the picture of aplastic anemia in the end stages. The roentgenographic findings of dense opaque bone establish the diagnosis if osteoplastic metastasis and Paget's disease are excluded. Multiple myeloma is recognized by the increased plasmacytes in the marrow, Bence Jones protein in the urine and punched out areas in the bones.

In rare instances of myelophthisic anemia the color and volume indexes may be high in which case differentiation from pernicious anemia may be difficult until the sternal marrow has been examined. The saturation index may be low if chronic hemorrhage co-exists.

4 *Anemias Associated with Splenomegaly*³—This is unquestionably a miscellaneous group but the large spleen is the chief clinical sign and the error of grouping them together under the term splenic anemia is not rare. Therefore, their differential diagnosis is discussed here. They all have in common a variable degree of normocytic anemia, leukopenia and thrombopenia and an enlargement of the spleen of moderate to great degree with moderate anisocytosis, poikilocytosis and increase in the immature red cells.

(a) *Banti's disease*⁴ (rare)—This usually begins at about 10 to 25 years of age, runs its course in 2 to 10 years and is rarely seen after 35. It is characterized by anemia with simple leukopenia and moderate thrombopenia followed by hemorrhages from varices in the stomach and esophagus, cirrhosis of the liver, and finally ascites (transudate in character). The anemia is moderate in severity until after the

¹ Ciordano A. S. and Blum L. L. Acute Hemolytic Anemia (Lederer Type). *Am J M Sc* 194, 311-326 (Sept.) 1937.

² Lamb F. H. and Jackson R. L. Osteopetrosis (Marble Bone Disease). *Am J Clin Path* 8, 255-272 (May) 1938.

³ MacCarty W. C. Chronic Splenomegaly. *Arch. Int. Med* 41, 536-547 (April) 1928.
McMichael J. Splenic Anaemia. *Edinburgh M J* 4, 97-116 (June) 1935.

⁴ Howells L. Treatment of Splenic Anaemia and Banti's Syndrome. *Lancet* 1, 1320-1324 (June 11) 1938.

hemorrhages begin, when it may become extreme or fatal. If the hemorrhages are allowed to continue, low saturation, volume, and color indexes result. All the laboratory changes characteristic of Laennec's cirrhosis occur in the later stages. Of these, the increased urobilinogenuria is the most important. The lymph nodes are not enlarged which differentiates it from Hodgkin's disease and aleukemic leukemias. In the early stages it is differentiated from familial acholuric jaundice by the leukopenia, low icterus index, normal or increased resistance of the red cells to laking by hypotonic saline, and normal average size of the red cells.

In the later stages, it is differentiated from familial cirrhosis of the liver¹ by the earlier age incidence, familial occurrence, more rapid course, and the earlier development of liver changes and ascites in the latter. The blood findings are the same.

Primary tuberculosis of the spleen² and schistosomiasis involving the spleen may give a laboratory and clinical picture indistinguishable from Banti's disease.

Portal thrombosis or thrombosis of the splenic vein give pictures almost identical with Banti's disease, but changes in the liver are not demonstrable, the history of a cause for thrombosis may be elicitable, and they may occur at any age. Most cases reported as simple or idiopathic splenomegaly belong in this group.

Banti's disease is differentiated from Laennec's cirrhosis by the earlier age incidence and the onset of splenic enlargement and anemia long preceding the evidences of cirrhosis. The blood picture in Laennec's cirrhosis is very similar to that of the later stages of Banti's disease.

(b) *Cirrhoses of the Liver*—Laennec's cirrhosis is the commonest cause of the blood picture described for Banti's disease and a similar blood picture may occur in any form of cirrhosis producing portal hypertension. However, the physical and laboratory signs of cirrhosis of the liver precede the onset of the anemia.

(c) *Lipoid Histiocytoses*—(1) Gaucher's disease³ (rare). The blood findings are identical with those of early Banti's disease, but the anemia is usually less marked. It differs in the earlier and later age incidence (2 to 40), longer course (20 years), the greater enlargement of the spleen, the presence of characteristic wedge shaped elevations of the conjunctiva near the cornea, and of bone changes which are radiographically demonstrable. Hemorrhages are less apt to occur and ascites is absent throughout, although the liver is usually enlarged.

It seems to be a congenital abnormality of lipid metabolism which results in the loading of all cells of the reticulo endothelial system with finely divided lipid droplets.

(2) Niemann Pick's Disease. This is extremely rare and is differentiated by the early age incidence (under 4), rapid course (rarely over 2 years), and enormous enlargement of liver and spleen. The blood changes are similar. In both the diagnosis can be established definitely by finding the characteristic foam cells in material obtained by sternal or splenic puncture.

¹Gunn Francis D. Familial Juvenile Cirrhosis of the Liver. Arch Path and Lab Med 1: 527-541 (April) 1926.

Bridgeman M. L. and Robertson T. D. Familial Juvenile Cirrhosis of the Liver. Am J Dis Child 43: 1155-1161 (May) 1932.

²Price A. E. and Yardine R. L. Primary Tuberculosis of the Spleen. Its Clinical Resemblance to Banti's Disease. Ann Int Med 4: 1574-1583 (June) 1931.

³Welt Sara Rosenthal N. and Oppenheimer B. S. Gaucher's Splenomegaly. J A M A 92: 637-644 (Feb 23) 1929.

(3) *Xanthomatosis or Hand Schuller Christian Disease* This may give either a normocytic anemia of the splenomegalic type or of the myelophthisic type The disease is characterized by large defects in the bones exophthalmos, and the syndrome of diabetes insipidus The spleen is enlarged and sternal or splenic puncture may reveal foam cells¹ The disease is due to infiltration of cells of the monocyte series with cholesterol esters

(d) *Hodgkin's Disease and Lymphosarcoma* (lymphoblastoma²)—These two conditions are so closely related that they can best be discussed together Both affect all ages and both sexes but Hodgkin's disease is commoner in young adults (15 to 35) and lymphosarcoma in the middle aged (35 to 60) Both are characterized by localized enlargement of lymphatic tissue which later becomes more generalized Moderate enlargement of the spleen usually occurs The blood findings are extremely variable and not diagnostic Moderate to marked anemia with normal indexes simple leukocytosis with evidences of somewhat increased erythropoiesis and red cell destruction, are most common, but leukopenia or leukemoid (rare and then only terminally in lymphosarcoma) pictures may occur and lymphocytosis monocytosis or eosinophilia are common Gordon observed that intra cerebral inoculation of ground lymph nodes from Hodgkin's disease produced encephalitis in animals but this has been proved to be a test for eosinophils³ rather than a specific test for Hodgkin's disease

Lymphosarcoma has a tendency to more invasive growth and a more rapid course Differentiation can be made with certainty only by removal of a lymph node for biopsy The relatively normal sternal marrow will differentiate these diseases from leukemias which they clinically resemble

The anemia is due in part to invasion of the marrow as in myelophthisic anemias and in part to splenomegaly but unknown factors apparently are the major cause of anemia in this group Deep roentgen therapy will relieve pressure symptoms from enlarged lymph nodes and spleen but the diseases are 100 per cent fatal with our present knowledge

Other causes of anemia often associated with moderate or extreme splenomegaly are malaria syphilis, subacute bacterial endocarditis, Felty's syndrome⁴ the leukemias, and kala azar These do not belong to this group and are usually easily differentiated if considered

5 *Endocrine Hypofunction*—Myxedema is usually associated with a moderate to severe anemia with normal indexes and may give a regenerative blood picture simulating that of pernicious anemia Pernicious anemia and myxedema may occur in the same patient Anemia occurs in Addison's disease but is the exception rather than the rule The mechanism of the production of anemia is unknown More study of such cases and of polycythemia rubra vera may aid in the determina-

¹ Erf L A Studies of Gaucher Cells by the Supravital Technique Am J M Sc 195 144-150 (Feb) 1938

² Minot G R and Isaacs R Lymphoblastoma (Malignant lymphoma) J A M A, 86 1185-1189 and 1265-1270 (April 17 and 24) 1926

Roth Grace M and Watkins C H The Leukocyte Picture in Hodgkin's Disease Ann Int Med 9 1365-1372 (Apr) 1936

³ Turner J C Jackson H Jr and Parker F Jr The Etiologic Relation of the Eosinophil to the Gordon Phenomenon in Hodgkin's Disease Am J M Sc 195 27-32 (Jan) 1938

⁴ Price A E and Schoenfeld J B Felty's Syndrome Report of a Case with Complete Postmortem Findings Ann Int Med 7 1230-1239 (Apr) 1934

tion of what constitutes the regulatory mechanism for the level of the erythrocytes in the blood

6 Malignant Tumors—Anemia is usually absent in the malignant tumors which do not bleed and do not involve the bone marrow, and may be absent at death. Hence, anemia in patients who have malignant tumors should suggest one of these two complications

The mechanism of the production of such anemias as do occur is insufficiently studied, but is probably related to both blood destruction and marrow inhibition by toxic absorption products of necrosis and secondary infection

Hypochromic microcytic anemia is the commonest form and results from chronic hemorrhage and deficient absorption of iron due to achlorhydria, poor appetite or vomiting. Normocytic anemias occur in those tumors which metastasize to bones or are secondarily infected

7 Aplastic Anemias¹—The idiopathic form is a rare disease of young adults which progresses without remission to a fatal termination. A tendency to hemorrhages into the skin and from the mucous membranes usually dominates the clinical picture. Hence, it must be differentiated from aleukemic leukemia and idiopathic purpura hemorrhagica

The icterus index is low (rarely normal or elevated due to absorption of internal hemorrhages). Urobilinogen is decreased in the stools and urine (except during absorption of hemorrhages)

The red cell count and hemoglobin progressively and rapidly decrease
Granulopenia is constant and often extreme. Lymphocytes are relatively increased but absolutely decreased. Cells of the granulocyte series are few or absent and old forms are relatively increased

In the stained smear, the erythrocytes appear normal or show poikilocytosis only. Polychromatophilia, basophilic stippling, and nucleated red cells are not found

Reticulocytes are decreased or absent

Nucleated cells are scarce or absent in the sternal marrow and those remaining are chiefly lymphocytes. The total nucleated cell count is usually less than 6,000

The platelet count is greatly decreased and hemorrhagic symptoms are the rule. The bleeding time is markedly prolonged and the clot time may be increased. Clot retraction is delayed or absent

The diagnosis should not be made in the absence of leukopenia, thrombopenia, nor if reticulocytes, polychromatophilic cells, or nucleated red cells are found. The aplastic anemias are excessive roentgen

¹ Rhoads C. P. and Miller D. K. Histology
Arch. Path. 26: 648-663 (Sept.) 1938

In Aplastic An

therapy, to exposure to radio active substances, external, or internal as in the occupational poisoning of workers with luminous paint, or to poisoning with benzol, organic arsenicals, or other benzol ring drugs differ only in having a determinable cause, and in recovery if that cause is removed sufficiently early

Most of the patients with a blood picture of aplastic anemia prove on examination of sternal marrow to have aleukemic leukemias. It is possible that the so called "idiopathic" form is the end stage of an aleukemic lymphocytic leukemia since cases have been reported and I have seen one case in which marrow studies were typical of acute lymphocytic leukemia at one time and later of aplastic anemia. At necropsy no evidence of leukemic infiltration in any organ was found and the marrow was typically aplastic. Some have used the term aplastic anemia for any anemia which presents the blood picture described irrespective of the marrow picture, but it seems wiser to limit the term to that group of cases in which the marrow is truly aplastic.

*F Anemias of Infancy and Childhood*¹—There are certain forms of anemia that need to be considered only in children. Most of the anemias described above as they occur in adults with the exception of pernicious anemia may develop in children but produce somewhat different blood pictures than they do in adults.

A condition known as von Jaksch's anemia or pseudoleukemia infantum is usually included in this group. The criteria given are enlarged spleen with anemia and leukocytosis, often of high degree. The blood picture shows an unusual number of nucleated red cells including karyoblasts (megaloblasts) and other evidence of rapid red cell formation. The differential white cell count is variable either lymphocytosis or neutrophilia occurring with a considerable increase in immature leukocytes. It may simulate leukemia or pernicious anemia. It is undoubtedly not an entity but rather represents the reaction of the infantile hematopoietic system to any severe cause of anemia of the blood destruction or blood loss type. It is rare after three years of age. The common causes of this syndrome are rachitis (associated infection rather than the vitamin deficiency per se) congenital syphilis and other infections.

1. *Erythroleukoblastosis*² (erythroblastosis)—This was formerly called icterus gravis neonatorum or universal edema of the new born. Many of these cases were probably included under the term von Jaksch's anemia. The disease is characterized by evidences of an extremely rapid rate of cell formation and an extremely rapid rate of red cell destruction. Nucleated erythrocytes may be more numerous in the blood than in any other disease. Immature leukocytes are also frequently seen. The icterus index is high and urobilinogen excretion is increased. It is

¹ Josephs H. W. *Anaemia of Infancy and Early Childhood*. *Medicine* 15: 307-451 (Sept.) 1936.

² Diamond L. K., Blackfan K. D. and Baty J. M. *Erythroblastosis Fetalis and its Association with Universal Edema of the Fetus*. *Icterus Gravis Neonatorum and Anemia of the Newborn*. *J. Ped.* 1: 269-309 (Sept.) 1932.

LABORATORY DIAGNOSIS

characterized clinically by jaundice dating from birth macrocytic anemia enlargement of the spleen and radial striations in the bones of the skull, demonstrable in roentgenograms. It may be evident at birth or be first noticed within the first 3 weeks of life. Multiple foci of hematopoiesis in the spleen, liver and other organs are the characteristic pathology. The marrow shows extreme hyperplasia (a) *Universal edema of the newborn* is apparently a more severe form of the same disease in which death usually occurs shortly after birth or the infant is stillborn. The pathology is the same.

(b) *Congenital anemia* is apparently a milder form of the same disease in which clinical jaundice is absent and nucleated erythrocytes are not so abundant in the blood, but normocytic or macrocytic anemia dates from birth.

It has been suggested¹ that this group of conditions is due to an anomaly in the placenta permitting interchange of fetal and maternal blood with resultant production of hemolysins for the infant's erythrocytes in the blood of the mother. These hemolysins enter the fetal circulation through the communication in the placental circulation during fetal life and are excreted in milk. If this theory is correct infants with this disease should not be breast fed. Another theory is that the disease is hereditary.² All three forms of the disease may occur in children of the same family but several normal children may be born before any show evidence of the disease. Those with universal edema nearly all die before birth or shortly after. Those with erythroleukoblastosis die if untreated but may survive if not breast fed and if given frequent transfusions. Those with congenital anemia may recover without treatment and nearly all recover with treatment.

Erythroleukoblastosis is differentiated from Cooley's anemia by the early age of onset, from leukemia by the presence of icterus and the scarcity of granuloblasts (myeloblasts). Otherwise the blood and marrow pictures closely simulate those of granulocytic (myelogenous) leukemia. It is differentiated from the anemia of congenital syphilis by the negative serologic tests in mother and infant and the absence of stigmata of congenital syphilis.

2 *Cooley's Anemia (thalassemia)*.³—This is a familial disease occurring in children of Mediterranean parentage. The anemia usually develops between 6 months and 2 years of age and the clinical and laboratory pictures are similar to that described for erythroleukoblastosis except for the later age of onset and the fact that the anemia is more often normocytic. The pathology is similar with multiple foci of hematopoiesis scattered through the organs. The prognosis is 100 per cent fatal within a few months to a few years. The later age of onset and nationality differentiate it from erythroleukoblastosis. Cases of this disease were undoubtedly included formerly under the term of Von Jaksch's anemia.

3 *Congenital Syphilis*.—This may produce severe normocytic anemia with enlargement of the spleen and great numbers of immature erythrocytes and leukocytes in the blood. The anemia may date from birth or develop within the first 6 months. The pathology is similar to that described for erythroleukoblastosis except for the later age of onset and the fact that the anemia is more often normocytic. The pathology is similar with multiple foci of hematopoiesis scattered through the organs. The prognosis is 100 per cent fatal within a few months to a few years. The later age of onset and nationality differentiate it from erythroleukoblastosis. Cases of this disease were undoubtedly included formerly under the term of Von Jaksch's anemia.

¹ Hellman L. M. and Hertig T. A. Pathological Changes in the Placenta Associated with Erythroblastosis of the Fetus. *Am J Path* 14 111-120 (Jan) 1938.
² Macklin Madge T. Erythroblastosis Foetalis. A Study of Its Mode of Inheritance. *Am J Dis Child* 53 1245-1267 (May) 1937.
³ Whipple G. H. and Bradford W. L. Mediterranean Disease—Thalassemia (Erythroblastic Anemia of Cooley). Associated Pigment Abnormalities Simulating Hemochromatosis. *J Pediat* 9 279-311 (Sept) 1936.
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few years of life. Positive serologic tests for syphilis in mother or infant and stigmata of congenital syphilis establish the diagnosis. Many recover with prompt adequate antisyphilitic treatment.

4 **Goat's Milk Anemia.**—Infants or children who have been kept for some period of time on an exclusive diet of goat's milk develop a macrocytic anemia with a blood picture similar to that described for pernicious anemia. The diagnosis is easy from the history and blood examination. The therapy is administration of antipernicious anemia principle and a diet suitable to the infant's age.

5 **Hypochromic Microcytic Anemia.**—This is usually due to keeping the infant too long on an exclusive milk diet or to failure to give extra iron to a premature infant. Most of the storage of iron in the liver occurs in the last month of fetal life and premature infants require added iron during the period of milk feeding to prevent development of such an anemia.

6 **Normocytic Anemias.**—Any of the normocytic anemias may occur but the commonest are those due to infection, the myelophthisic anemias and the splenomegalic anemias. The treatment is the same as that described for adults, but the differential diagnosis from leukemia may offer more difficulty because in young children a greater number of immature leukocytes may be present in the blood or marrow as a response to infection or marrow invasion than in the same conditions in adults. All of the conditions must be excluded before it is safe to make a diagnosis of leukemia in an infant under 3 years of age unless great numbers of blast cells are found in the blood or marrow or the total nucleated cell count in the blood is over 100,000 per c. mm.

VI TOTAL PLASMA AND BLOOD VOLUME DETERMINATION¹

This procedure is not used much as yet in diagnosis but it is of considerable importance in many lines of research and knowledge of the various volume states characteristic of the different conditions aids materially in interpreting other clinical and laboratory data. It should be done in all cases in which the diagnosis of polycythemia vera is considered. It should be determined under basal conditions (see page 103 for directions to patient).

A **Normals.**—These may be expressed in cc. per kilogram of body weight or in cc. per square meter of body surface. The latter is probably more reliable in adults; the former in children.

In adults of both sexes the plasma volume ranges from 42 to 65 cc. (average 51 cc.) per kilogram of body weight or from 1600 to 2250 cc. (average 1900 cc.) per square meter of body surface. In newborn infants the plasma volume per kilogram of body weight is the same as in adults but it averages about 10 cc. per kilogram higher in infants from 12 days to 10 months of age (range 38 to 72 cc.). The values have returned to the adult standard by four years of age. Expressed in terms of square meters of body surface, however, the values are distinctly lower for infants (750 cc. at birth, 1100 cc. at one year) and for children (increase to 1375 cc. by the twelfth year) than for adults.

For total blood volume in adults the figures are 72 cc. to 105 cc. (average 88 cc.) per kilogram of body weight or 2700 cc. to 4000 cc. (average 3300 cc.) per square

¹ Rowntree L. C. Brown C. F. and Roth Grace E. *The Volume of the Blood and Plasma in Health and Disease*. Pp. 210. W. B. Saunders Company Philadelphia 1929.

Darrow D. C. Soule H. C. and Buckman J. E. *Blood Volume in Normal Infants and Children*. J. Clin. Invest. 5: 243-258 (February) 1928.

meter of body surface The results tend to be about 5 per cent higher in men than in women, because of the greater volume of red cells The total blood volume is 105 to 195 cc (average 150 cc) per kilogram in the newborn (the higher value is due to the large number of red cells) The figures are 70 to 125 cc (average 100 cc) per kilogram in infants from 15 days to one year of age In older children the values range from 65 to 105 cc (average 80 cc) per kilogram of body weight Per square meter of body surface in children over one year of age the figures are 1400 cc to 2500 cc (average 1900 cc), tending to be a little lower in the earlier age groups and higher in the older children The total volume of circulating blood is thus about 400 cc at birth, 300 cc at 1 month 400 cc at 6 months 700 cc at 1 year, 1000 cc at 2 years, 1500 cc at 6 years, and 2000 cc at 10 years In women the average is about 5000 cc (range 4000 cc to 6000 cc) and in men the average is about 6000 cc (range 4500 cc to 7500 cc)

B Increased Blood Volume—1 **Polycythemia Vera**—This is by far the most important cause of alterations in blood volume The total volume is very seldom within normal limits and is often twice the normal value and sometimes even higher It is at times increased when the red cell count and hemoglobin percentage are within normal limits Hence it is of definite diagnostic value Both plasma and cell volume are increased but as a rule the increase in the latter is proportionately greater

2 **Other Causes**—In leukemias, especially the granulocytic (myelogenous) type in secondary erythrocytoses, and in the later months of pregnancy, values average higher than in normals, but only a few of the results are above the upper limits of normal variation Plasma volume is chiefly affected in leukemia and pregnancy, cell volume in the erythrocytoses Plasma volume is above the normal average but near the upper limits of the normal range in all types of splenomegaly

In hypertensive cardiovascular renal disease the blood volume is within normal limits and tends to be low rather than high

C Decreased Blood Volume—1 **Acute Hemorrhage and Shock**—Decreased blood volume may reach a high grade but the volume determinations are not necessary for the diagnosis The knowledge that this decreased volume occurs furnishes a definite indication for therapy and explains why the red cell count and hemoglobin do not correctly indicate the degree of anemia in the first 24 to 48 hours after an acute hemorrhage

2 **Myxedema**—The average plasma and total blood volume are near the lower limits of normal Some anemia is usually present which is more marked than the red cell counts or hemoglobin estimations would suggest

3 **Anemias**—In all types of anemias except those associated with splenomegaly and with glomerular or diffuse nephritis there is a tendency for the total blood volume to be slightly below and the plasma volume to be above the average normal The degree of increase in plasma volume is greater the greater the degree of red cell deficiency and is probably compensatory in an effort of the body to maintain a normal blood volume

4 **Glomerulonephritis and Chronic Diffuse Nephritis with Edema**—The plasma volumes are normal and the red cells decreased resulting in a slight decrease in total blood volume which is seldom outside normal limits Other types of edema (nephroses cardiac edema) show values for total blood and plasma volume which are within normal limits but which average slightly higher than in normals

5 Obesity.—In the obese the values are normal in terms of body surface but low per kilogram of body weight.

6 Dehydration.—This may occur from many causes including vomiting, diarrhea, excessive sweating, fluid deprivation and severe burns. The decrease may be extreme and affects chiefly the plasma volume.

III DIFFERENTIAL DIAGNOSIS OF POLYCYTHEMIAS¹ OR ERYTHROCYTOSES

The essential feature of these conditions is an increase above the normal limits in red cells or hemoglobin. In adults and children over 3 months of age a count over 7.0 million for males and over 6.5 million for females would strongly suggest one of these conditions. Polycythemia may be simulated by dehydration (decreased plasma volume), as after severe burns, shock, vomiting, diarrhea, or prolonged abstinence from fluids. Thus high counts are common in patients with dysentery or cholera. In these cases the fact that the blood is more concentrated than normal is the significant point since there is no increase of the total number of red cells in the body. On the other hand, it may be masked by increased plasma volume. Hence, when this diagnosis is suspected even though counts within the upper limits of normal are found a blood volume determination is indicated.

1 *Polycythemia Rubra Vera*,² *Vaquez* or *Osler's Disease*³.—This is a clinical entity of unknown etiology characterized by a reddish cyanosis, enlargement of the spleen, increased total blood volume and a marked erythrocytosis usually associated also with a simple leukocytosis and a thrombocytosis. Red cell counts as high as 16.0 million per cubic millimeter have been reported but seem open to question since the cells would have to be very small to have any fluid present with them. The hemoglobin and cell volume are not increased to the same degree as the red cell count; hence the color and volume indexes tend to be low but the saturation index is normal. Immature leukocytes and red cells are common and the blood picture may suggest granulocytic (myelogenous) leukemia. Some cases⁴ terminate as granulocytic (myelogenous) leukemia. These may be differentiated by sternal puncture which gives the typical findings of granulocytic (myelogenous) leukemia while in the other cases of polycythemia vera the marrow picture is normal. They are sometimes called erythroleukemia.

Therapy with phenylhydrazine should be controlled by frequently repeated routine blood examinations to prevent excessive red cell destruction until a dose has been established which will just maintain the erythrocyte count within normal limits. An increase in the leukocytosis after phenylhydrazine is to be expected.

¹ Harrop G. A. Polycythemia. *Medicine* 7: 201-244 (Aug.) 1928.

² Haden R. L. The Red Cell Mass in Polycythemia in Relation to Diagnosis and Treatment. *Am. J. M. Sc.* 196: 403-502 (Oct.) 1938.

Rosenthal N. and Bassen I. A. Course of Polycythemia. *Arch. Int. Med.* 62: 903-917 (Dec.) 1938.

³ See reference p. 170 by Rabschtein Robbin.

⁴ Klumpp T. G. and Hertig A. T. Eryth. Cases Presenting Aspects of Both Diseases.

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The increase in red cells is apparently due entirely to increased marrow activity. Red cell destruction is increased rather than diminished. The basal metabolism and blood uric acid are elevated.

A benign familial form of polycythemia has been reported.¹

B Erythrocytoses—1 **Erythrocytoses Secondary to Anoxemia**.—This type occurs in the following conditions and may be extreme. Increase in leukocytes and platelets and evidences of rapid red cell regeneration are confined largely to the period of onset. Increase in blood volume is present only in the more marked cases and is seldom as great as in polycythemia vera. Enlargement of the spleen is absent.

(a) *Decreased Oxygen Tension in the Alveolar Air*—Residence at high altitude is the clinical cause, but the same result occurs on experimental lowering of the oxygen tension. The increase is about 50 000 cells per 1000 feet and cell counts up to 8 000 000 have been observed. The increase begins at once due to contraction of the spleen and is maintained with evidence of rapid regeneration during the stage of increase. It returns to normal on raising the oxygen tension as by return to sea level.

(b) *Insufficient Aeration of Blood Due to Pulmonary Disease*²—This may occur in extensive pulmonary fibrosis (tuberculosis, pneumoconiosis), emphysema, pulmonary atelectasis (unilateral pneumothorax), or in primary arteriosclerosis or syphilis of the pulmonary arteries (rare). Members of this group have been described under the term Ayerza's disease or black cardinals because of the extreme bluish black cyanosis which at once differentiates them from the red cyanosis of the group above described. Clubbing of the nails is common in this group.

(c) *Insufficient Aeration of the Blood Due to Cardiac Disease*—Erythrocytoses up to 11 000 000 per cubic millimeter occur in the cyanotic group of congenital heart disease particularly pulmonary stenosis, the tetralogy of Fallot, and other cases in which there is a shift of blood from the right side of the circulation to the left without passage through the lungs. In many cases in group (b) the associated right heart hypertrophy, dilatation and finally failure, secondary to obstruction in the pulmonary circuit (cor pulmonale), undoubtedly play a part. The majority of cases of pulmonary arteriosclerosis are secondary to such obstruction and the resultant hypertension in this circuit. This, also, occurs in some cases of mitral stenosis and erythrocytosis may occur with it but is seldom as marked as in congenital heart disease. Clubbing of the fingers or pulmonary osteoarthropathy is rarely absent.

(d) *Chemical Causes*—Chronic carbon monoxide poisoning belongs in this group. Continuous exposure to very small concentrations of carbon monoxide over a long period of time is necessary and the polycythemia disappears within a few weeks after removal from exposure. No symptoms occur in this form of carbon monoxide poisoning.

2 **Erythrocytoses of unknown mechanism** (probably bone marrow irritation?). These are relatively uncommon causes.

(a) *Diseases of the Bone Marrow*—Polycythemia is reported in rare cases of granulocytic (myelogenous) leukemia and multiple myeloma.

¹ Spodaro A. and Forkner, C. E. Benign Familial Polycythemia. Arch Int Med 52: 593-602 (Oct.) 1933.

² Barker N. W. Polycythemia Vera and Chronic Pulmonary Disease. Arch Int Med 47: 94-103 (Jan.) 1931.

(b) *Stimulation of Marrow(?)*—It occurs in rare instances following acute hemorrhage, in paroxysmal hemoglobinuria, or hemophilia

(c) *Poisons*—Phosphorus arsenic, gum shellac and various anilin dyes may produce it

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CHAPTER VII

DISORDERS OF THE LEUKOPOIETIC SYSTEM WITH ESPECIAL REFERENCE TO INFECTIOUS DISEASES AND LEUKEMIAS

The histogenesis, embryology and anatomy of the leukopoietic system are reviewed in conjunction with that of the erythropoietic system in Chapter VI

I PHYSIOLOGY

A Stimuli—What are the fundamental adequate stimuli for the increased formation and liberation of the different types of white cells? For neutrophils, these may be adenine sulphate, nucleotide, or perhaps a foreign protein to which the individual is not allergic, for eosinophils, a foreign protein to which the individual is allergic, for monocytes, particulate matter in the blood stream, for lymphocytes, invasion of lymphoid tissue, but none of these is proved beyond dispute, in none is the mode of action understood, and certainly there are other stimuli

B Depressants—Do specific depressants of blood cell formation occur or is there merely an absence of the specific stimulant in the various types of deficient formation of a particular cell? Probably both occur. Certain chemical poisons such as benzol and aminopyrine have been shown to be depressants in some persons, but the bacterial toxins having this action have not been identified

C The Duration of Life—What is the duration of life or mode of destruction of white cells? Loss from the gastrointestinal tract, in saliva and in pus is obvious, and disintegration within the blood stream is evident, especially in leukemias. Studies by the marrow culture method¹ indicate a duration of life of the neutrophil lobocytes (polymorphonuclears) of 48 to 90 hours, average 60 hours, of the eosinophils of 8 to 12 days, and of the basophils of 12 to 15 days. The lymphocytes of the blood are able to divide by amitotic division so data on their duration of life was not obtainable by the marrow culture technic. Other data by this method suggest a duration of life if amitotic division is prevented of 24 to 48 hours

II TOTAL, DIFFERENTIAL AND ABSOLUTE LEUKOCYTE COUNTS OF THE BLOOD

A Methods—**I Leukocyte Counts**—The leukocyte counts are subject to the same types of counting errors as the erythrocyte counts. Criteria for estimating the accuracy of a given count and of

¹ Osgood E. E. Culture of Human Marrow. Length of Life of the Neutrophils, Eosinophils and Basophils of Normal Blood as Determined by Comparative Cultures of Blood and Sternal Marrow from Healthy Persons. J. A. M. A. 109: 933-937 (Sept. 18) 1937

determining the significance of an apparent difference in any of these counts have been given in Table 8

2 **Wright's Stain**—Wright's stain, if performed as directed on page 477, is recommended in preference to Giemsa's stain or other Romanowsky stains. It has many advantages over the supravital technic for routine clinical use

3 **Supravital Staining**¹—Many studies which are of importance have been based on the study of living cells but for clinical purposes it has few advantages over Wright's stain and is much more troublesome to do. It is somewhat easier to identify monocytes by this technic and harder to identify some other cells than with Wright's stain. Its chief advantage is that it is possible to gauge to some extent the functional activity of the cells studied. Interpretation of the significance of the different cell types is as yet the same as that given for Wright's stain. Much research is yet to be done with this method before its full clinical possibilities can be delimited. Lymphocytes are easily overlooked in supravital counts

B Normals²—1 **Total White Cell Counts**—The normal figures for the different groups are given in Table 7 and in Fig 7. There are no sex differences in the total white cell count. Note the great variations with age and also that the changes occur at different ages than for the red cell factors. Even in adults, the figures usually given as normal (5,000 to 10,000) include only 80 per cent of healthy persons. In other words, one healthy person in every 5 examined will have a

¹ Sabin Florence R. Studies of Living Human Blood Cells. Bull. Johns Hopkin Hosp. 34: 277-288 (Sept.) 1923

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² Osgood I. F. Baker R. L. Brownlee Inez F. Osgood Mable W. Ellis D. M. and Cohen W. Total Differential and Absolute Leukocyte Counts and Sedimentation Rates of Healthy Children Four to Seven Years of Age. Am. J. Dis. Children to be published.

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in identification of the leukocytes. Note that the normal values given below are entirely different for children and adults and that a differential count which would be perfectly normal for an adult would indicate a definite increase in neutrophils in a child. There are no sex differences in differential or absolute leukocyte counts.

Unavoidable errors in the differential cell count due purely to chance¹ are often not sufficiently considered in interpretation. The accompanying chart (Fig. 8) will show at a glance the significance of a

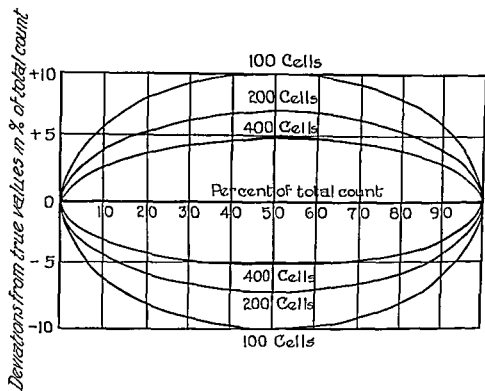


FIG. 8—Chart showing the unavoidable error due to chance in the differential cell count (Modified from Barnett)

report. It is calculated so that the curves correspond to a deviation of plus or minus 2 standard deviations from the average, in other words, so that there is only about one chance in 20 that the actual count is outside of the range indicated. The method of using the chart is as follows: suppose the count reported is 50 per cent neutrophils based on a count of 100 cells. Note that the 50 per cent line intersects the 100 cell line at a plus or minus 10 per cent. Therefore, there is only one chance in 20 that the actual count is less than 40 per cent or more than 60 per cent. If 400 cells are counted, there is only one chance in 20 that the count is over 55 or under 45 per cent. Its chief value is in

¹ Barnett, C. W. The Unavoidable Error in the Differential Count of the Leukocytes of the Blood. *J. Clin. Invest.* 12: 77-85 (Jan.) 1933.

determining whether a variation from day to day in the count is significant. For example, if the lymphocyte count is 70 per cent, based on a count of 100 cells, and the next day it has risen to 85 per cent, it is probably a true increase, but if the second count were 77 per cent, one could not be sure it was not a difference due to chance alone.

(a) *Neutrophil Lobocytes (Segmented Neutrophils)*—See Table 7. These make up 33 to 75 per cent (average 54 per cent)¹ of the total number of white cells in the blood of adults 20 years of age and over. For adolescents, 15 to 19 years of age the range² is 25 to 70 per cent with an average of 48 per cent. For children 4 to 14 years of age, and probably also for infants, 10 days to 4 years of age, the range is 16 to 60 per cent with an average of 38 per cent. In the newborn the lobocyte (segmented neutrophil) count averages 60 per cent on the first day and rapidly drops to 50 per cent on the third day, 40 per cent the fourth day, and, thereafter, averages about 38 per cent with a 95 per cent range of 16 to 60 per cent as in older children. Counts as high as 75 per cent neutrophils in the first 4 days of life may not be abnormal. Counts under 35 per cent in the first 2 days are uncommon.

Ninety-five per cent of healthy persons over 2 days old will show an absolute value for lobocytes (segmented neutrophils) of 1500 to 7500 per c mm with an average of 4,000 in each age group except the newborn in the first 2 days of life which average 9000 on the first day and 6,000 on the second day and children 8 to 14 years of age in whom the average is 3,250 per c mm.

(b) *Neutrophil Rhabdocytes ("Staff" Cells)*—These have been included with the polymorphonuclear neutrophils in the past in differential counting but information of considerable diagnostic value is obtained by separating them from the neutrophil cells containing a segmented nucleus. They make up from 0 to 5 per cent of the white cells in the blood of adults, and a slightly higher percentage during childhood. This may be as high as 17 per cent during the first year of life and 25 per cent the first day of life. In older children and adults a count of over 10 per cent may be considered abnormal.

Ninety-five per cent of healthy persons will show an absolute value for rhabdocytes ("staff" cells) of 0 to 1500 per c mm.

(c) *Eosinophils*—In persons over 14 years of age, the average is 2.0 per cent and the range is from 0 to 6 per cent. In children 4 to 13 years

¹ The probable explanation for the higher figures (64 per cent) usually given is that most of the previous work has been done on patients who were thought not to have infectious diseases rather than on perfectly healthy individuals such as the subjects of the series on which these figures are based.

² All the ranges given for differential cell counts include 95 per cent of the results.

of age, the average is 2.8 per cent with a range from 0 to 8 per cent. These figures apply to younger children, too.

Ninety-five per cent of healthy persons will show an absolute value for eosinophils of 0 to 600 per c mm if under 14 years of age and 0 to 400 if more than 14 years of age.

(d) *Basophils*—These are quite rare in normal blood, ranging from 0 to 2 per cent and averaging 0.5 per cent.

Ninety-five per cent of healthy persons will show an absolute value for basophils of 0 to 200 per c mm.

In ordinary differential counting all stages of eosinophils and of basophils are grouped together. It is only when very high counts occur or when marrow or leukemic blood is being counted that it is necessary to separate the various immature cells from the lobocytes (polymorphonuclears).

(e) *Lymphocytes*—In adults 20 years of age or over these make up from 15 to 60 per cent (average 38 per cent) of the total white cells. In adolescents 15 to 19 years of age the average is 42 per cent with 95 per cent of the results between 22 and 62 per cent. In children 4 to 14 years of age, the average is 48 per cent with a range from 20 to 70 per cent and these figures probably apply also to children from 5 days to 4 years of age. In the newborn the lymphocyte percentage averages 30 per cent on the first day, 35 per cent on the second day, 40 per cent on the third day, and 45 per cent on the fourth day.

Ninety-five per cent of healthy persons 20 years of age and over will show an absolute value for lymphocytes of 1000 to 4500 per c mm with an average of 2750. In persons 15 to 19 years of age the values are 1500 to 5000, average 3250. In children 8 to 14 years of age the values are 1500 to 6500, average 4000. In children 4 to 7 years of age and probably also in younger children the values are 1500 to 8500, average 5000.

These normal lymphocytes are divided by some into large, medium, and small lymphocytes, but no sound morphologic, histogenetic, or clinical reason has been advanced for this subdivision. In fact, all the available evidence suggests that they are normal variants of the same cell form. Further, the misuse of the term large lymphocyte for certain of the normal lymphocytes causes confusion with the prolymphocyte. The latter has a definite diagnostic significance and is definitely larger than the neutrophils of normal blood. The term lymphocyte should be used when referring to the normal cell and the term prolymphocyte should be reserved for the immature cell.

(f) *Monocytes*—These make up from 0 to 9 per cent, average 4 per cent, of the white cells in the blood of healthy persons 14 years of age and

over, 0 to 7 per cent, average 3 per cent, in the blood of children 4 to 13 years of age, and about 5 per cent with a range from 0 to 12 per cent of the cells in the blood of infants from birth to 4 years of age

Ninety five per cent of healthy persons in all age groups will show an absolute value for monocytes of 0 to 800, average 300, per c mm

(g) *Disintegrating Cells*—These cells are probably not artifacts made in smearing but remnants of dead cells. They should not be omitted from the differential count as a large number of disintegrating cells is strongly suggestive of a diagnosis of leukemia and failure to include them may give an erroneous impression of the true incidence of other cell types. Normal values for all ages and both sexes are 0 to 12 per cent, average 5 per cent, with absolute counts of 0 to 1200 per c mm, average 400

C *Physiologic Variations*—The total leukocyte count is usually less than 15,000 per c mm

1 *Simple Leukocytosis*—This should be differentiated from the neutrophilia of infections. In this form of leukocytosis the increase in count affects all cell types and eosinophils, basophils and monocytes do not disappear from the blood as they do in neutrophilia. The neutrophils may increase somewhat in percentage but are rarely over 80 per cent

(a) *Muscular Activity*—After very severe exercise counts as high as 35,000 have been reported. Less severe exertion does not increase the count so much, but counts as high as 15,000 are not uncommon. Complete cessation of muscular activity (basal conditions) reduces the white cell count somewhat below the usual figures for normal. Sometimes an increase in the lymphocytes instead of neutrophils is responsible for the increase. The most probable explanation for the effect of muscular activity on the white cell count is a contraction of the spleen, but a washing out of white cells by the increased rate of blood flow is a possible factor

(b) *Diurnal Variations*¹—A digestive leukocytosis has long been considered to occur, but rather conclusive evidence is now available to show that digestion has no effect on the leukocyte count and that

¹ See Garrey and Bryan previously cited on page 231

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changes formerly thought to be due to digestion are merely diurnal variations which occur whether food is ingested or not, and are more closely related to mental and physical activity than to anything else. The variations in one day in one person tend to cover almost the entire normal range and the total count and percentage of neutrophils tend to be highest in the afternoon. These daily variations make it difficult or impossible to attribute much value to tests such as the leukopenic index¹ in which multiple counts over a short period of time are compared unless the differences are great. In all probability many of these variations are actually due to the unavoidable error in counting methods as illustrated in Table 8.

(c) *Pregnancy, Labor, and the Puerperium*²—In the last month of pregnancy, a leukocyte count just over or just under the upper limits of normal is the rule. During labor the average rises to about 18,000 in primipara and about 15,000 in multipara. Counts may be as high as 30,000. In the first day or two of the puerperium, this high level is maintained and then it falls gradually to reach the normal level ten to fourteen days post partum in uncomplicated cases. An increase in the percentage of neutrophils and in the relative proportion of young forms is associated.

(d) *Physical Measures*—Cold bathing, massage, or cutaneous effect of ether may cause a temporary rise in the white cell count to as high as 14,000 to 25,000. High fever, artificially induced, is associated with a fall in leukocytes which is followed in 6 to 9 hours by a marked rise.

(e) *Pain*—Pain, alone, is capable of increasing the leukocyte count to 20,000.

(f) *Drugs*—Simple leukocytosis may occur after administration of adrenalin,³ ethereal oils, liver extract or phenylhydrazine.

D Leukocytoses and Leukopenias—It has been common in the past to classify the leukocytoses solely by the cell type increased but it is just as valuable to pay attention to the other cell types which are decreased or absent. The classification here used has proved the most useful in my experience and was first published in the Atlas of Hematology.⁴

¹Loveless Mary Dorfman R. and Downing Lillian A. Statistical Evaluation of the Leukopenic Index in Allergy. *J Allergy* 9 321-344 (May) 1938.

²Jarcho J. Changes in the Leucocytes during Pregnancy and the Puerperium. A Review of the Literature and an Analysis of Fifty five Cases. *Am J Obst and Gynec* 17 13-20 (Jan.) 1929.

³Lucia S. P. Leonard M. E. and Falconer E. H. The Effect of the Subcutaneous Injection of Adrenalin on the Leukocyte Count of Splenectomized Patients And of Patients with Certain Diseases of the Hematopoietic and Lymphatic Systems. *Am J M Sc* 194 35-43 (July) 1937.

⁴Osgood E. E. and Ashworth Clarence M. *Atlas of Hematology*. Pp 178-195. J. W. Stacey Inc. San Francisco 1937.

1 **Leukocytoses** —Leukocytosis is an increase in the total number of white cells per cubic millimeter. It is usually due to an increase in only one type but may be due to an increase in several types of cell. Therefore, the relative proportions of the types of cells present are usually altered. The type of leukocytosis takes its name from the cell most markedly increased. An increase in the neutrophils is called a neutrophilic leukocytosis or a neutrophilia, in lymphocytes, a lymphocytosis etc.

There may be a change in the relative proportion of any cell type without its total number exceeding that normally present. The total number per c mm present in a patient's blood may be calculated by multiplying the percentage of this cell type by his total count. If the total number present is more than that of normal blood, it is called an *absolute* increase, if less than that normally present, it is called a *relative* increase. For example the maximum normal percentage of eosinophils in the blood of children is 8 per cent and the maximum absolute number is 600. If the patient's count is 10,000 with 10 per cent eosinophils, there is an absolute eosinophilia ($0.10 \times 10,000$ is 1000, which is more than 600). If the count is 5000 with 10 per cent eosinophils, there is a relative eosinophilia (10 per cent of 5000 is 500, which is less than 600).

(a) *Simple Leukocytosis* —This was formerly included with neutrophil leukocytosis but differs from neutrophilia in that eosinophils, basophils and monocytes do not disappear from the blood. Simple leukocytosis is defined as an increase in the total leukocyte count above the upper limits of normal for the age and sex group without disappearance of eosinophils, basophils or monocytes and without absolute or relative lymphocytosis. The neutrophil percentage is either normal or increased and the absolute number of neutrophils is often increased. This type of leukocytosis occurs with pain, muscular activity and the other disturbances of physiology listed as causes of leukocytoses and also in the following conditions: *amebic abscess, acidosis, anemia of acute hemorrhage, myelophthisic anemia, bee stings, burns, cholera, chorea, coccidioid granuloma, convulsions, coronary occlusion, dissecting aneurysm, eclampsia, exercise, familial hemolytic icterus, fractures, glanders, gonorrhea, internal hemorrhage, Hodgkin's disease, insanity, intestinal obstruction, leukemia, liver extract administration, lymphosarcoma, nephritis, Oroya fever, osteomyelitis, pain, periarthritis nodosa, phenylhydrazine administration, polycythemia rubra vera, pregnancy, puerperium, rabies, rat bite fever, relapsing fever, rheumatic fever, rickets, salpingitis*

*sarcoma scarlet fever, sickle cell anemia, skull fracture, smallpox*¹ *sub acute bacterial endocarditis syphilis, tetanus, tuberculosis, tularemia, typhus, undulant fever, and uremia* Counts within normal limits are common in all the above conditions and counts above 20,000 are uncommon except in acute hemorrhage, burns, cholera, leukemia, osteomyelitis, polycythemia, during the normal puerperium, and in rheumatic fever Hodgkin's disease scarlet fever and smallpox occasionally give counts over 20,000 Such a blood picture may also occur in the stage of recovery in most of the conditions typically producing a neutrophilia Multiple bee stings and smallpox may cause the appearance of many progranulocytes (promyelocytes) and granulocytes (myelocytes) in the blood, giving a leukemoid blood picture

(b) *Neutrophilic Leukocytosis or Neutrophilia*—This is an increase in the absolute number of neutrophilic cells in the blood It is nearly always associated with an increase in the proportions of immature forms, first those with few segments, then rhabdocytes (staff cells) and in the more severe grades, with the appearance of metagranulocytes (metamyelocytes) or granulocytes (myelocytes) in the blood The increase in the relative proportions of immature forms may also occur when the total number of neutrophilic cells is not increased or is even decreased, and is then of great diagnostic value The importance of this increase in immature forms has long (since 1904) been stressed by Arneth,² but his classification proved too complex to be widely used In 1912 V Schilling³ suggested a modification which is clinically practical Many others have since introduced workable classifications, but the valuable point in all is the same, i e., to separate the neutrophilic cells of normal blood and the leukocytoses into groups of greater and lesser degrees of maturity For practical purposes, this is most satisfactorily done by classifying the cells of the granulocyte series in the blood in the manner described in this book But the criteria for the different stages are somewhat different from those of Schilling and I agree with those who feel that the rhabdocytes (staff cells) are an immature cell rather than with Schilling's view that it is a degenerating

¹ Ikeda K The Blood in Smallpox During a Recent Epidemic Arch Int Med 37 660 (May) 1930

² Arneth J Die neutrophilen weissen Blutkörperchen bei Infektion krankheiten Pp 200 Gustav Fischer Jena 1904

Arneth J Die qualitative Blutlehre 4 vol 1920-1928 An enormous amount of material but so minutely classified as to be impractical for clinical purposes

³ Schilling V The Blood Picture Translated by Gradwohl R B H from the seventh and eighth German edition Pp 408 C V Mosby St Louis 1929 This book (preferably the original in German) should be consulted if a full exposition of Schilling's views on hematology is desired They stimulate thinking for one well founded in hematology but the beginner must constantly keep in mind while reading it that it expresses the views of one man some of which are not generally accepted

cell It is customary to speak of an increased proportion of the more immature forms as a "shift to the left," and of the more mature forms as a "shift to the right", but this nondescriptive terminology should be discouraged It is derived from the report blanks used by Germans for recording results of a differential count, the younger forms being tabulated to the left of the more mature forms A relative or absolute increase in the particular cell type or in immature or old forms is far more descriptive, and to be preferred

In all the conditions listed below as giving rise to a neutrophilia, an increase in the proportion of immature neutrophils also occurs

(1) *Acute Infectious Diseases*—Neutrophilia occurs in anthrax, bronchopneumonia, diphtheria, bacillary dysentery, all forms of acute meningitis including that due to the typhoid bacillus, lobar pneumonia and in typhoid fever in children Pneumonia is the most common infectious disease producing neutrophilia Acute gastroenteritis, impetigo contagiosa, Ludwig's angina, and streptococcic sore throat or tonsillitis may produce neutrophilia

(2) *Accumulations of Pus, Especially When That Pus Is under Pressure*—The commoner members of this group are appendicitis, salpingitis, otitis media, abscesses (except the tuberculous), acute cholecystitis, empyema, peritonitis, acute pyelitis, pyelonephritis, pyonephrosis, gas gangrene, mastoiditis, and acute sinusitis Any infection with staphylococci, streptococci, or the other pyogenic organisms may produce such a neutrophilia The degree of neutrophilia depends more on the pressure than on the quantity present Thus, a few drops of pus from the middle ear may give a higher leukocytosis than a large suppurating wound Thickly encapsulated collections of pus in which the bacteria are dead or quiescent usually give rise to no neutrophilia

(3) *Acute and Subacute Bacteremias Due to the Pyogenic Organisms*—Monocytes usually persist in the blood in this group

If a neutrophilia has been found, it should be followed by repeated total and differential white cell counts until the values have returned to normal In very acute conditions, such as appendicitis, hourly counts are desirable, and in all acute cases daily counts are indicated changing to biweekly, then weekly determinations in subacute stages and conditions A sedimentation rate determination will be found of value in most cases showing a neutrophilia

The maximum information can be derived from leukocyte counts only after very extensive clinical experience and study¹

¹ See the books by Pepper and Farley, Piney, Arnet, and Schilling elsewhere referred to and for interpretation of hematologic findings in children the book by Baar and Stransky cited at the end of this chapter Also

(c) *Eosinophilic Leukocytosis or Eosinophilia*¹—This is an absolute or relative increase in the eosinophilic cells. There may be no increase in the total count. The causes are

(1) *Parasitic Infection*—This should be thought of first when eosinophilia is found

Trichiniasis—This is the most common cause of a high absolute eosinophilia. The eosinophils may reach 80 per cent and are rarely less than 15 per cent except at the onset. The total white cell count is usually increased. Subclinical cases probably account for many cases of unexplained eosinophilia.

Intestinal Parasites—Any of the intestinal parasites may give rise to an eosinophilia which is usually slight (under 10 per cent) or moderate (10 to 30 per cent) in degree, but the total count is usually within normal limits, and eosinophilia is often absent.

Parasites of the Blood and Blood Forming Organs—Malaria, filariasis, trypanosomiasis, and kala azar are associated in some instances with eosinophilia of varying degree (usually slight).

(2) *Allergic Conditions*—It is probable that all causes of eosinophilia except the group due to bone marrow involvement belong in this group. The definitely proved members of this group are

True Bronchial Asthma—Eosinophils are found in large numbers in the sputum as well as in the blood. This is one of the common and important causes. Eosinophilia does not occur in so called cardiac asthma and is therefore of differential value. The eosinophilia is usually under 20 per cent and averages about 10 per cent.

Hay Fever

Urticaria and Angioneurotic Edema

Serum Sickness and after the Injection of Vaccines and Foreign Proteins of Various Types

Favism

Doan A. C. and Zerfas L. G. The Rhythmic Range of the White Blood Cells in Human Pathological Leucopenia and Leucocytic States with a Study of Thirty-two Human Bone Marrows. *J. Exp. Med.* 46: 511-539 (Sept.) 1927.

Cooke, W. E. and Ponder E. The Polynuclear Count. Pp. 80. Lippincott Philadelphia 1917.

Piney A. The Significance of the Polynuclear (Cooke) and Schilling Leucocyte Counts. *Quart. J. Med.* 22: 405-412 (April) 1929.

Reznikoff P. White Blood Cell Counts in Convalescence from Infectious Diseases. *Am. J. Med. Sci.* 184: 167 (Aug.) 1932.

Weiss A. The Staff Count. Its Importance in Acute Infectious Disease. *Arch. Int. Med.* 48: 399 (Sept.) 1931.

Medlar E. M. and Kastlin G. J. The Polymorphonuclear Count in the Tuberculous Blood Picture. *Am. J. Med. Sci.* 173: 824-834 (June) 1927.

¹Page I. H. Turner K. B. and Wilson J. H. The Clinical Significance of Eosinophilia on a General Medical Service. *J. Lab. and Clin. Med.* 13: 1109-1116 (Sept.) 1928.

(3) *Infectious Diseases*—Most of these conditions give simple leukocytosis without disappearance of eosinophils rather than a true eosinophilia. *Scarlet Fever*¹ is the only infectious disease in which eosinophilia occurs early with any high degree of constancy. It may occur early in *acute rheumatic fever* or *gonorrhea*, and a slight eosinophilia is the rule during convalescence from most febrile diseases. Its early appearance in a febrile disease has, thus, some diagnostic value and its later appearance may be regarded as a favorable sign. It occurs in some patients who have syphilis but this has no diagnostic value.

(4) *Certain Skin Diseases*—It depends more on the extent and severity of the destruction of the skin (area for absorption of foreign or altered proteins?) than on the nature of the disease. It is especially marked in those characterized by the formation of large blebs and in the exfoliative dermatitis of arsenic poisoning. Dermatitis herpetiformis may sometimes cause eosinophilia as high as 40 per cent with a total count up to 20 000.

(5) *Diseases of the Bone Marrow*

Granulocytic (Myelogenous) Leukemia and the Rare Eosinophilic Leukemia—The highest absolute counts of eosinophils occur in these conditions.

Carcinoma or Sarcoma of the Bone Marrow

Osteomyelitis—Think of this when neutrophilia, eosinophilia, and fever are associated.

Osteomalacia, rickets and polycythemia rubra vera are also occasionally associated with eosinophilia.

(6) *Poisoning with Certain Drugs*—Arsenic and camphor are the most important members of this group. It is not constant, but may reach a high level (59 per cent eosinophils with a white count of 30,500 in one case of arsenical dermatitis seen by the author). The usual finding in acute arsenic poisoning,² is a normal or slightly decreased total leukocyte count and a moderate eosinophilia (average 19 per cent, range 2 to 40 per cent in 5 cases studied).

(7) *Periarteritis Nodosa*. This disease usually gives a simple leukocytosis but may be associated with slight or severe eosinophilia.

(8) *Miscellaneous conditions* in which eosinophilia may occur but is inconstant and of little diagnostic value. Hodgkin's disease, pernicious anemia and malignant tumors. Eosinophilia is not common in patients with malignant tumors but occa-

¹Friedman S. Eosinophilia in Scarlet Fever. I. As a Diagnostic Aid. *Am J Dis Child* 49: 933-935 (Apr.) 1935. II. General Considerations. *Id.* 49: 1257-1263 (May) 1935.

²Lawson C. B., Jackson W. P. and Cattarachi C. H. Arsenic Poisoning. Report of Twenty-eight Cases. *J. A. M. A.* 83: 24-26 (July 4) 1925.

sionally due to involvement of bone marrow, absorption of foreign or altered proteins from ulcerated areas, or in tumors with metastases in the liver slight to extreme eosinophilia¹ may occur. Eosinophilia sometimes develops after raw liver therapy for anemia, after splenectomy, and in Addison's disease.

The demonstration of eosinophilia should cause one to search for intestinal parasites and ova, and for evidence of allergic reaction. If, in addition, abnormal white cells are present in the blood, disease of the bone marrow should be suspected. In some cases a thorough search has failed to reveal a cause, hence apparently not all the causes have been determined. The higher normal eosinophil percentage in young children must be considered in interpretation.

(d) *Basophilia*—An increase in basophils is rare and is not diagnostic of any disease except basophilic leukemia, but suggests bone marrow involvement. It may occur in any of the conditions listed under (5) above in the discussion of eosinophilia. It is rarely absent in granulocytic (myelogenous) leukemias. It also occurs in polycythemia rubra vera, Hodgkin's disease, anemias associated with splenomegaly, and in some chronic skin diseases, but is slight and inconstant in all. One case of true basophilic leukemia has been reported.

(e) *Lymphocytosis*²—Formerly many diseases were listed as causes of lymphocytosis but in most of these diseases the actual figures for lymphocytes were within normal limits or actually depressed and the increase in lymphocyte percentage was due either to failure to recognize the wide limits of normal lymphocyte percentage or to a decrease in cells of the granulocyte (myeloid) series. Most of the conditions which give normal lymphocyte percentages will be found under the category of simple leukopenia and those which produce a relative lymphocytosis due to decrease in the granulocytic (myeloid) cells are grouped together in the category of granulopenia.

The causes of absolute lymphocytosis are few.

Lymphocytic Leukemias—These give rise to an absolute lymphocytosis which may reach 1,000,000 cells per cubic millimeter and over 99 per cent lymphocytes. Less than 70 per cent lymphocytes is unusual. Immature lymphocytes are present in the acute and subacute cases. Lymphocytes may be normal in morphology and number in the blood in aleukemic forms.

¹ Currin H B. A Case of Marked Eosinophilia. Northwest Med 37: 296-297 (Sept) 1938.

² Whitney Caroline. Hyperplasia of Lymphoid Tissue and Lymphocytosis. Medicine 7: 1-30 (Feb) 1928.

Pertussis (whooping cough) ¹—Figures of over 100,000 cells per cubic millimeter, chiefly lymphocytes, have been reported, and counts of 15,000 to 40,000 with over 60 per cent lymphocytes are the rule in the paroxysmal stage. Earlier, the cell count may be normal.

Infectious Mononucleosis (glandular fever, acute and benign lymphadenosis) ²—A moderate leukocytosis with an absolute lymphocytosis and a considerable number of prolymphocytes is the usual finding. The prolymphocytes must not be confused with monocytes which are little if at all increased. This condition is differentiated from acute lymphocytic leukemia, which the blood picture may at first suggest, by the absence of anemia and of lymphoblasts which are characteristic of the latter and by the presence of a positive Paul and Bunnell test. The normal sized lymphocytes often show fenestrated nuclei ³.

(f) *Monocytosis* ⁴—This is usually only relative. It seems probable that the fundamental cause is particulate matter in the blood stream. It may occur in (1) *monocytic leukemia*, (2) *malaria* (differentiates from liver abscess), (3) *subacute bacterial endocarditis*, (4) *tuberculosis* ⁵ (during the stage of hematogenous extension), (5) *Hodgkin's disease* (inconstant), (6) *lymphosarcoma* (inconstant), (7) *cachexia of carcinoma*.

Slight monocytosis occurs late in the course of most infectious diseases. It may reach a considerable height in smallpox, typhus, and cholera.

It may be produced experimentally by infection with *B. monocytogenes* or injection of lipoids from tubercle bacilli or of tetrachlorethane.

Only in monocytic leukemia, subacute bacterial endocarditis and in chronic malaria is the monocytosis apt to be of diagnostic value. Much stress has been laid on the value of the leukocyte count in the diagnosis and prognosis of tuberculosis. It is true that the neutrophils increase with caseation and secondary infection and monocytes with hematogenous extensions, while lymphocytes tend to increase during healing stages, but so many other factors may influence these cells that

¹ Thelander H. E., Henderson H. G. and Kilgariff K. The Blood Picture in Pertussis. A Graphic Study. *J. Ped.* 2: 288 (March) 1933.

² Dolgopol Vera B. The Blood Picture in the Early Stages of Pertussis. *J. Ped.* 3: 367 (Aug.) 1933.

³ Gold A. E. and Bell H. O. Improvement in the Diagnosis of Whooping Cough. Correlation Between Sedimentation Rate and Cell Count of Blood on the Basis of Proved Cases. *Am. J. Dis. Child.* 52: 25-40 (July) 1936.

⁴ Downey H. and McKinley C. A. Acute Lymphadenosis Compared with Acute Lymphatic Leukemia. *Arch. Int. Med.* 32: 82-112 (July) 1923.

⁵ Sprunt T. P. Infectious Mononucleosis (Glandular Fever). *International Clinics* 3: 93-119 1933.

⁶ See reference 3 on page 251.

⁷ Doan C. A. and Wiseman B. K. The Monocyte Monocytosis and Monocytic Leukosis. A Clinical and Pathological Study. *Ann. Int. Med.* 8: 383-416 (Oct.) 1934.

⁸ See footnote 1 on p. 244.

the value of the decreasing $\frac{\text{lymphocyte}}{\text{monocyte}}$ ratio, the increasing $\frac{\text{neutrophil}}{\text{lymphocyte}}$ ratio, and the increasing proportion of neutrophil cells of immature type as criteria of a bad prognosis has probably been over emphasized. Almost any type of white cell count is compatible with any type of tuberculosis, and while averages differ, individual variations are so great that the counts have very little diagnostic value, and should be used in prognosis only as secondary in importance to the clinical and roentgenographic data. The differing points of view will be found presented in more detail in the references¹ and the articles included in their bibliographies.

(g) *Plasmacytosis*—A few cells of the plasmacyte series are present in normal blood but they are too scarce to be found frequently in ordinary differential counting. Plasmacytosis of 2 to 20 per cent is found in German measles and measles in some cases of multiple myeloma and in some of the myelophthisic anemias. A great increase occurs in the rare plasmacytic leukemia.

2 *Leukopenias*—(a) *Simple Leukopenia*—This is defined as a decrease in the total leukocyte count with a percentage differential count within normal limits. The conditions listed under this heading were formerly included under the heading of relative lymphocytosis. Actually these diseases usually give a normal differential cell count with, occasionally, an increased proportion of rhabdocytes (staff cells) and either a normal or low total leukocyte count. Of course, the term leukopenia applies only if the total count is below normal limits for a

¹ Medlar E. M. An Evaluation of the Leucocytic Reaction in the Blood as Found in Cases of Tuberculosis. *Am Rev Tuberc* 20 312-346 (Sept.) 1929.

Flinn J. W., Flinn R. S., and Flinn, Z. M. Correlation of Blood Counts in One Hundred and Fifty Clinical Cases of Tuberculosis and Underlying Pathological Changes as Shown by Serial X Ray Films. *Am Rev Tuberc* 27 488 (May) 1933.

Cunningham R. S. and Tompkins Edna H. The White Blood Cells in Human Tuberculosis as Studied by the Supravital Technique. *Am Rev Tuberc*, 17 204-239 (March) 1928.

Farley D. L. St. Clair H. and Reisinger J. A. The Normal Filament and Nonfilament Polymorphonuclear Neutrophil Count. Its Practical Value as a Diagnostic Aid. *Am J Med Sci* 180 336-344 (Sept.) 1930.

Reilly, W. A. Behavior of Monocytes in Tuberculosis in Children. *Am Rev Tuberc* 25 178 (Feb.) 1932.

Sabin Florence R. Cellular Studies in Tuberculosis. *Am Rev Tuberc* 25 153-171 (Feb.) 1932.

Morris W. H., and Wilson G. C. The Leucocytic Blood Picture in Active and Inactive Tuberculosis. A Comparison of Differential Blood Counts Made During Clinical Activity with Others Made After Clinical Arrest. *Am Rev Tuberc* 33 66-74 (Jan) 1936.

Stasney J. and Feldman W. H. The Character of the Leucocytic Response to Tuberculin in Sensitized Calves. *Am J M Sc* 195 20-27 (Jan.) 1938.

Sullivan M. and Jones P. H. Diagnosis of Early Tuberculosis. The Value of Monocytic Lymphocytic Index Determined by Supravital Technique Before and After the Administration of Tuberculin. *Am J M Sc* 185 762-768 (June) 1933.

Sweany H. C. Strom Ingrid and Cannemeyer Wilma. A Composite Blood Chart as an Aid in the Control of Treatment of Tuberculosis. *Am Rev Tuberc* 35 129-133 (Jan.) 1937.

person of the patient's age group. Most of them are conditions affecting the lymphatic tissues, chronic infections or diseases associated with hypertension in the portal circulation or involving the spleen. In none of these diseases is leukopenia always present. In none of them is the neutrophil count usually under 20 per cent as it usually is in the group of granulopenias. The conditions which may cause simple leukopenia are *macrocytic anemias*, *splenomegalic anemias*, *Banti's disease*, *cachexia* and *malnutrition*, *chickenpox*, *cirrhoses*, *dengue*, *Felty's syndrome*,¹ *Gaucher's disease*, *granuloma inguinale*, *Hodgkin's disease*, *lymphosarcoma*, *influenza*, *kala azar*, *leprosy*, *malaria*, *measles*,² *mumps*, *Niemann Pick's disease*, *pappataci fever*, *paresis*, *psittacosis*, *poisoning with the heavy metals*, *lead*, *bismuth*, or *mercury*, *relapsing fever*, *schistosomiasis*, *sprue*, *Still's disease*, *thrombosis or stenosis of the portal vein*, *tuberculosis*, *typhoid fever*, *undulant fever*, *Vincent's angina*, *xanthomatosis*, and *yellow fever*. Since, in all of these, counts within normal limits are common a simple leukopenia suggests that one of these conditions is present but a normal count does not exclude the diagnosis.

(b) *Granulopenia* ³—The term granulopenia seems more descriptive than the old term relative lymphocytosis for the conditions included in this group since the major change is a decrease in neutrophils, eosinophils and basophils. The total leukocyte count is usually between 100 and 2,000. The total lymphocyte count is often actually decreased. The causes are *agranulocytosis*, *idiopathic or secondary aplastic anemia*, and *aleukemic or subleukemic leukemias*. The disappearance of granulocytes in all of these conditions may result in the development of a gangrenous stomatitis or pharyngitis due to invasion by the organisms already present. The finding of a granulopenia constitutes a definite indication for sternal puncture and examination of the sternal marrow.

(1) *Agranulocytosis* ⁴—This disease is sometimes called agranulocytic angina, malignant neutropenia or essential granulopenia. It is characterized by a primary decrease in mature cells of the granulocyte (myeloid) series in the blood and marrow. It is due to an idiosyncrasy to drugs containing the benzol ring including aminopyrine, sulfanilamide,

¹ Craven E B Jr Splenectomy in Chronic Arthritis Associated with Splenomegaly and Leukopenia (Felty's Syndrome) J A M A 102 823 (March 17) 1934

² Benjamin B and Ward Sylvia M Leukocytic Response to Measles Am J Dis Child 44 921-963 (Nov) 1932

³ Mettler S R and Olson H T The Clinical Significance of Leucopenia with Special Reference to Idiopathic Neutropenia Ann Int Med 6 855-868 (Jan) 1933

⁴ Kracke R R Relation of Drug Therapy to Neutropenic States J A M A 111 1255-1259 (Oct) 1938

Beck Regina C Benign and Malignant Neutropenia Present Status of Knowledge of This Condition with Report of Four Cases Arch Int Med 52 239-287 (Aug) 1933

Roberts S R and Kracke R R Further Studies on Granulopenia with a Report of Twelve Cases Ann Int Med 8 129-147 (Aug) 1934

dinitrophenol, benzol itself, and the organic arsenicals. Such idiosyncrasy occurs in a relatively small percentage of persons but since the disease is often fatal it is important that other drugs be substituted for these if equally effective compounds are available and that all patients receiving these drugs have daily routine hematologic examinations on the first three days after beginning therapy and at relatively frequent intervals thereafter. Absence of anemia and thrombopenia differentiate agranulocytosis from aplastic anemia but a sternal puncture is usually necessary to differentiate it with certainty from aleukemic or subleukemic leukemias although the history of exposure to a drug containing the benzol ring suggests the diagnosis of agranulocytosis. The most important point in treatment is early discovery and removal of the cause. Administration of blood transfusions, pentose nucleotide and liver extract have been followed by recovery.

C. The Significance of Immature Leukocytes in the Blood or Marrow¹—Study plates IV and V and their legends. Tables 38 to 42 and the illustrations in the Atlas for details of the morphology of the immature leukocytes.

1. Granuloblasts² (myeloblasts)—A count of over 20 per cent granuloblasts (myeloblasts) in the sternal marrow or the appearance of granuloblasts (myeloblasts) in the blood almost certainly indicates the presence of granulocytic (myelogenous) leukemia. The greater the percentage of granuloblasts (myeloblasts) among the cells present, the more acute the leukemia is likely to be. In some cases of acute leukemia, almost all the cells present are granuloblasts (myeloblasts) and in such cases the inexperienced are likely to mistake them for cells of the lymphocyte series because, superficially, the granuloblast (myeloblast) resembles the lymphocytes more than it does the mature granulocytic (myeloid) cell. This mistake will not be made if the chromatin structure of each cell examined is noted and the importance of fine chromatin structure is clearly understood. A mature lymphocyte always has coarse chromatin in clumps whereas the granuloblast (myeloblast) has a fine lace net like structure. The size of the granuloblast (myeloblast) which is usually larger than a neutrophil lobocyte (polymorphonuclear) as a rule will differentiate it from a lymphocyte.

¹ This material has been reproduced by permission of the copyright owners from Osgood E. E. and Ashworth Clarice M. Atlas of Hematology. Pp 16-71. J. W. Stacey, Inc. San Francisco 1937.

² Downey, H. The Myeloblast—Its Occurrence under Normal and Pathological Conditions and Its Relations to Lymphocytes and Other Blood Cells. *Folia haematologica* 34: 65-89 and 145-187 (June and August) 1927.

Downey, H. The Occurrence and Significance of the Myeloblast under Normal and Pathologic Conditions. *Arch Int Med* 33: 301-313 (March) 1924.

However, in the rare instances when small granuloblasts (myeloblasts) are present, the chromatin structure is the only point of differentiation from the lymphocyte. If the cells present are all stem cells there is no way of conclusively classifying the leukemia as granulocytic (myeloid), lymphocytic or monocytic. In nearly all cases the classification may be made by prolonged search which will reveal some progranulocytes (promyelocytes), prolymphocytes or promonocytes either in the blood or marrow.

2 **Progranulocytes A (promyelocytes II)**—The finding of progranulocytes A (promyelocytes II) in the blood or more than 100 per cent in the marrow should always suggest a diagnosis of granulocytic (myelogenous) leukemia. Progranulocytes A (promyelocytes II) are usually the predominant cell in the blood and marrow in this disease. In acute granulocytic (myelogenous) leukemia most of the cells are likely to be granuloblasts (myeloblasts) and progranulocytes (promyelocytes), whereas in chronic granulocytic (myelogenous) leukemia the progranulocytes A (promyelocytes II) are associated with granulocytes (myelocytes), metagranulocytes (metamyelocytes) and rhabdocytes (staff cells). A few progranulocytes A (promyelocytes II) may appear in the blood in pernicious anemia, erythroleukoblastosis, polycythemia rubra vera, smallpox, and myelophthisic anemias such as result from osteomyelitis, metastases of malignant tumors to bone and multiple myeloma. A few progranulocytes (promyelocytes) may also appear in the blood in unusually severe infections of the type usually resulting in a neutrophilic leukocytosis. In this same group of conditions the progranulocyte (promyelocyte) count in the sternal marrow is usually 50 to 100 per cent.

3 **Neutrophil Progranulocytes S (promyelocytes I)**—The appearance of neutrophil progranulocytes S (promyelocytes I) in the blood or an increase to a count of over 100 per cent in the marrow strongly suggests the diagnosis of granulocytic (myelogenous) leukemia. If they are increased in the marrow and no abnormal cells are found in the blood the diagnosis is aleukemic granulocytic (myelogenous) leukemia. These cells are not commonly seen in acute granulocytic (myelogenous) leukemia but are often very numerous in chronic and subacute granulocytic (myelogenous) leukemia. Occasionally a neutrophil progranulocyte S (promyelocyte I) may be found in the blood of patients with pernicious anemia, polycythemia rubra vera, erythroleukoblastosis, smallpox, acute osteomyelitis and malignant tumors involving the bone. In rare instances a neutrophil progranulocyte S (promyelocyte I) may appear in the blood of patients with overwhelming infection.

4 Neutrophil Granulocytes (myelocytes)—The appearance of appreciable numbers of neutrophil granulocytes (myelocytes) in the blood or an increase to over 150 per cent in the marrow strongly suggests the diagnosis of granulocytic (myelogenous) leukemia. A few granulocytes (myelocytes) may, however, appear in the blood in very severe infections of the type associated with a neutrophilic leukocytosis. Neutrophil granulocytes (myelocytes) may also be found in the blood and increased in the marrow in pernicious anemia, polycythemia rubra vera, erythroleukoblastosis, smallpox, acute osteomyelitis and malignant tumors invading the marrow such as multiple myeloma or metastatic carcinoma or sarcoma.

5 Neutrophil Metagranulocytes (metamyelocytes)—These cells may appear in small numbers in the blood in any condition associated with neutrophilic leukocytosis but if they constitute more than 50 per cent of the leukocytes a serious prognosis is indicated. A count of 200 per cent or more neutrophil metagranulocytes (metamyelocytes) strongly suggests a diagnosis of chronic granulocytic leukemia. A few metagranulocytes (metamyelocytes) may appear in the blood and they may be increased in the marrow in pernicious anemia, polycythemia rubra vera, erythroleukoblastosis, smallpox, acute osteomyelitis, and if malignant tumors such as multiple myeloma or metastatic carcinoma or sarcoma invade the marrow.

6 Neutrophil Rhabdocytes (staff cells)—An increase in neutrophil rhabdocytes (staff cells) in the blood may occur in any severe infection but occurs most commonly in the more virulent infections commonly associated with a neutrophilia. They may also be increased in the blood or marrow in absolute numbers and occasionally in relative per cent in chronic granulocytic (myelogenous) leukemia. A neutrophil rhabdocyte (staff cell) count of over 200 per cent in the blood indicates a severe infection and if the count exceeds 500 per cent in any disease other than malaria the prognosis is very grave. Some German authors speak of an increase in rhabdocytes (staff cells) as a shift to the left because of the position in which they tabulate these cells in the differential count on the laboratory forms. Giant rhabdocytes (staff cells) are not infrequently found in the blood or marrow in chronic granulocytic (myelogenous) leukemia or in macrocytic anemias, and some authors consider them diagnostic of pernicious anemia. Rhabdocytes (staff cells) are also increased in the blood in polycythemia rubra vera and in myelophthisic anemias.

7 Lymphoblasts—The occurrence of lymphoblasts in either the blood or sternal marrow is almost pathognomonic of acute lymphocytic

leukemia although some of the more mature types of lymphoblasts may be found in the blood and marrow in infectious mononucleosis. Since lymphoblasts are morphologically indistinguishable from granuloblasts (myeloblasts), monoblasts or plasmablasts, if cells resembling these are found in the blood or constitute more than 20 per cent of the cells seen in the marrow, it is certain that the patient has acute leukemia. The type of leukemia is determined by the other cells present in the blood or increased in the marrow.

8 Prolymphocytes—An occasional prolymphocyte may sometimes be found in the blood of patients with chronic lymphocytic leukemia, in the blood of normal infants, or in the blood of a person who has been receiving roentgen ray or radium therapy, but only in acute lymphocytic leukemia or infectious mononucleosis do prolymphocytes appear in the blood or sternal marrow in appreciable numbers. Not all authors agree that prolymphocytes are intermediate in maturity between lymphoblasts and lymphocytes. Wiseman believes that the maturity of the lymphocytes should be judged by the degree of basophilia of the cytoplasm rather than from size. He believes that the deeper the blue color the less mature is the cell.

(a) *Peroxidase Test*—This is indicated in any case in which difficulty arises in differentiating progranulocytes (promyelocytes) from prolymphocytes. This difficulty is most apt to occur in acute leukemias or infectious mononucleosis.

(1) *Interpretation*—Unfortunately this does not differentiate the granuloblasts (myeloblasts) from lymphoblasts but all other granulocytic (myeloid) cells show the peroxidase reaction. If large cells with round nuclei showing this reaction are present in the blood of a case of leukemia it is probable that the leukemia is of the granulocytic (myelogenous) type. As acute leukemias occur in which the preponderating cell is the granuloblast (myeloblast) absence of peroxidase granules in the large cells is a point in favor of lymphocytic leukemia but this does not exclude the possibility of its being a granulocytic (myelogenous) leukemia.

9 Monoblasts—Monoblasts probably occur normally in the marrow and possibly in the spleen but they are present in such small numbers and so closely resemble the granuloblasts (myeloblasts) that they do not appear in the differential cell count of normal sternal marrow. They are not found in the blood or lymph nodes of healthy persons. The presence of monoblasts in the blood or of appreciable numbers in the sternal marrow occurs only in monocytic leukemias. They are most numerous in the acute form and may not appear in the blood at all in the chronic form.

10 Promonocytes—Promonocytes are the type cells of monocytic leukemia and if found in the blood or in appreciable numbers in the marrow a diagnosis of this condition is justified. They constitute a

larger proportion of the cells of the monocyte series in the acute and subacute forms than in the chronic form of monocytic leukemia

11 Plasmablasts—Plasmablasts are increased in the marrow in multiple myeloma and plasmacytic leukemia. They may occur in the blood in either of these conditions but are numerous only in plasmacytic leukemia

12 Proplasmacytes—Proplasmacytes are increased in numbers in the blood and marrow in plasmacytic leukemia, multiple myeloma, German measles and, to a lesser extent, in measles, leukemias and chronic infections

13 Plasmacytes ¹—These cells are markedly increased in the blood and sternal marrow in plasmacytic leukemia, German measles and some cases of multiple myeloma. In most cases of multiple myeloma the increase is only in the sternal marrow but it is sufficiently constant and marked so that the author has correctly diagnosed two cases of multiple myeloma on the basis of this finding before other characteristic signs appeared. A moderate increase in plasmacytes in the blood or marrow may be found in measles and many other acute and chronic infections. The accumulations of plasmacytes in tissues which are chronically infected as in syphilis, or sinusitis, are even more constant. Many authors believe the plasmacytes are formed locally in the perivascular tissues but it seems more probable that a few plasmacytes and proplasmacytes migrate from the blood stream and proliferate both by mitotic and amitotic division. It is commonly thought that plasmacytes develop from lymphocytes but I have never found any evidence of this from my studies of multiple myeloma, plasmacytic leukemia or marrow or blood cultures. It seems certain that they are a distinct and separate line of cells

14 Mitosis in Cells—It has been shown by marrow culture experiments that all of the -blast cells and pro cells are capable of mitotic division and typical mitoses are seen normally in the marrow but in the blood only in leukemias. Amitotic division occurs in the normal lymphocyte, in the plasmacyte, proplasmacyte, and in the erythrocyte series in the karyocyte (pronormoblast) and prokaryocyte (erythroblast)

The immature cells of the eosinophil and basophil series have not been described since they differ from the corresponding neutrophil only

¹Michels N A. The Plasma Cell. A Critical Review of Its Morphogenesis, Function and Developmental Capacity under Normal and under Abnormal Conditions. Arch Path 11: 775-793 (May) 1931

Osgood E E and Hunter W C. Plasma Cell Leukemia. Folia Haemat. 52: 369-383 1934

in the granules and since they appear in blood only in the conditions listed as causes of eosinophilia or basophilia

F Anomalies of Leukocyte Morphology—1 **Pelger's Nuclear Anomaly**¹—This is a rare familial condition in which most of the neutrophils, eosinophils and basophils in normal blood without evidence of infection resemble rhabdocytes (staff cells) and the lobocytes (polymorphonuclears) rarely have more than two lobes. Its only significance is that it should be differentiated from an increase in immature cells due to infection.

2 **Polylobocytes (macropolycytes)**²—These are very large neutrophils with many nuclear lobes in the lobocyte (polymorphonuclear) stage and very large nuclei often branching around a clear area and then joining again. These cells may be seen in the blood and marrow in pernicious anemia and other macrocytic anemias. Some believe them diagnostic of these conditions. They may occur, however, in granulocytic (myelogenous) leukemia and in patients with extreme leukocytosis. Finding of these cells should suggest one of these diagnoses.

3 **Fenestrated Nuclei in Lymphocytes**³—Holes in the nuclei of lymphocytes and prolymphocytes which may appear as sharply demarcated clear spaces or more commonly as diagonal pale areas corresponding to holes through the nucleus at an angle to the line of vision are present in the blood of most patients with infectious mononucleosis. In some stages of the disease they may be abundant but are usually scarce. Finding of typical fenestrations in freshly drawn blood should always suggest this diagnosis. They have been seen in the nuclei of a few patients with chronic lymphocytic leukemia but only if the blood has been allowed to stand for some time before smears are made.

4 **Artefacts**—Fragments of the cytoplasm of the progranulocytes (promyelocytes) are often seen in the marrow and may be found in the blood of patients with granulocytic (myelogenous) leukemias. These fragments somewhat resemble malaria parasites but do not contain pigment, are not located inside of the erythrocytes and comparison with the cytoplasm of the progranulocytes (promyelocytes) should readily indicate their source.

¹ Tileston W. Familial Shift to the Left of the Leukocytes (Pelger's Nuclear Anomaly of the Leukocytes) with Report of a Case. *Ann Int Med* 11: 675-681 (Oct) 1937.

² Cooke W. E. The Macropolycyte. *J Lab and Clin Med* 19: 453 (Feb) 1934.

Jones O. P. Origin of Neutrophils in Pernicious Anemia (Cooke's Macropolycytes). *Biopsies of Bone Marrow*. *Arch Int Med* 60: 1002-1015 (Dec) 1937.

³ Osgood E. E. Fenestration of Nuclei in Lymphocytes. A New Diagnostic Sign in Infectious Mononucleosis. *Proc Soc Exper Biol & Med* 33: 218-219 (Nov) 1935.

G Prognostic Significance of Neutrophil Changes ¹—*In general the per cent of neutrophils, the toxic changes in neutrophil morphology, and the degree of increase in the immature forms are indications of the severity of the infection, and the total white cell count is an indication of the resistance of the individual* A decrease in the total count with a persistently high or increasing percentage of neutrophils, particularly the immature forms, is an unfavorable sign An overwhelming infection with any of the organisms which ordinarily lead to a neutrophilic leukocytosis may result in a relative neutrophilia with a normal or decreased total leukocyte count If, with such low counts, the neutrophilia is absent and only a preponderance of immature neutrophils is present, the prognosis is still worse It is in these cases in which a neutrophilic leukocytosis is expected but not found in which the separation of the rhabdocytes ("staff" cells) and other immature neutrophils from the lobocytes (segmented forms) is of most value Malaria is the chief exception to the rule that a rhabdocyte (staff cell) count of over 50 per cent indicates a grave prognosis

Indexes are available for comparing the total increase in leukocytes with the increase in neutrophils An increase of 5 points in the neutrophil percentage is usually stated to be associated with an increase of 5000 in the total leukocyte count, with 10,000 leukocytes and 75 per cent neutrophils as the base line, if the resistance of the patient is keeping pace with the severity of the infection A proportionately high total count would be a favorable point in forming the prognosis and vice versa This is of some value, if the age variations in the leukocyte count are kept in mind and other factors are also given due consideration

In children, of course, a neutrophilic percentage which might be normal in the adult may constitute a definite neutrophilia, and, on the other hand, an increase in immature forms has not as serious a prognostic import as an equal increase would have in an adult

Certain changes in the morphology of neutrophils have been shown to indicate a grave prognosis These changes (Plate VI) are basophilia of the cytoplasm, toxic granules, and the appearance of vacuoles The cytoplasm of the neutrophils is normally lilac in color like the granules, but paler In seriously ill patients, it may be a deep blue The normal neutrophil granules are fine, uncountably numerous, and lilac staining Toxic granules are relatively few in number, large in size, and violet or purple in color Only an occasional vacuole is seen

¹Harkins H N The Present Status of Blood Examinations in the Diagnosis of Surgical Infections with a Study of 27 Indices of Infection Reported in the Literature Surg Gyn and Obst 59 48-61 (July) 1934

Meyer H F The Prognostic Significance of the Leukocyte Count in Pneumonia of Children Am J Med Sci 181 245 (Feb) 1931

in a normal neutrophil. Vacuoles may be very numerous in the neutrophils of seriously ill patients. If these changes are graded on the scale of 1 to 4 plus, it will be found that less than 10 per cent of patients who show 3 to 4 plus changes live longer than a week.¹ Rosenthal and Sutro² divide the number of neutrophils showing toxic changes by the total number of neutrophils and multiply by 100 to get the degeneration index, a persistent degeneration index of 100 often presaging a fatal outcome.

III THE SEDIMENTATION RATE OF THE RED CORPUSCLES³

This test is so simple and so valuable if properly interpreted that it should be done routinely on all patients examined. It should be regarded as a somewhat different and sometimes more sensitive criterion of bodily reaction to injury than are fever and leukocytosis. Hence, its chief value is not in differential diagnosis, but in following the progress of the individual case. A normal rate does not exclude disease, but a rapid rate even in a patient thought to have hysteria or with no complaints is a valuable and easily secured indication for further study.

A large number of methods have been proposed for this determination, all of which gives results of clinical value. The Cutler,⁴ Linzenmeier,⁵ Wintrobe⁶ and Westergren methods are the ones most commonly used. The modified Westergren method given on page 501 has all the advantages of these methods and is much more rapid and convenient. The results are not quantitatively transferable from one method

¹ Osgood E. E. Palmer A. and Pollard W. T. Death Accurately Predicted from Leukocyte Morphology. To be published.

² Rosenthal N. and Sutro C. J. The Blood Picture in Pneumonia with Special Reference to Pathological Changes in Neutrophils. *Am J Clin Path* 3: 181-197 (May) 1933.

³ Kugel M. A. and Rosenthal N. Pathologic Changes Occurring in Polymorphonuclear Leukocytes During the Progress of Infections. *Am J Med Sci* 183: 657 (May) 1932.

⁴ Sutro C. J. Cytoplasmic Changes in Circulating Leukocytes in Infection. *Arch Int Med* 51: 747-753 (May) 1933.

⁵ Fahræus R. The Suspension Stability of the Blood. *Physiol Rev* 9: 241-274 (April) 1929.

⁶ Lundgren R. Study of the Sedimentation of the Blood Corpuscles. *Acta med Scandinav* 67: 63-104 1927.

⁷ Rourke Dorothy M. and Plass I. D. An Investigation of Various Factors which Affect the Sedimentation Rate of the Red Blood Cells. *J Clin Invest* 7: 365-386 (August) 1929.

⁸ Jeterman M. C. and Seegar S. J. Sedimentation Reaction in Children. *Am J Dis Child* 37: 693-730 (April) 1929.

⁹ Lefkowitz M. Die Blutkörperchensenkung. Erfahrungen und Bericht über die Literatur der letzten drei Jahre. Pp. 30. Urban and Schwarzenberg. Berlin 1932.

¹⁰ Bannick F. C. Crege R. O. and Guernsey C. M. The Erythrocyte Sedimentation Rate. The Adequacy of a Simple Test and Its Practical Application to Clinical Medicine. *J A M A* 109: 1257-1263 (Oct. 16) 1933.

¹¹ Cutler J. W. The Practical Application of the Blood Sedimentation Test in General Medicine. Observations Based Upon Approximately 5000 Patients Over a Period of Six Years. *Am J Med Sci* 183: 643 (May) 1932.

¹² Wintrobe M. M. and Landsberg J. W. A Standardized Technique for the Blood Sedimentation Test. *Am J M Sc.* 169: 102-115 (Jan.) 1935.

to another, but the degree of increase in rate will be roughly proportional in the different methods. Hence, though I give figures only for the recommended method, all conditions giving rapid rates with this method will show comparable increases in rate with other methods. The forty five minute reading by the preferred method and the one hour reading by the original Westergren technic will correspond fairly closely. Corresponding values for the Cutler and Linzenmeier methods may be calculated from the data of Greisheimer, Treloar and Ryan.¹

The rate is affected by room temperature² and by any alteration in plasma viscosity, or in numbers, size, or hemoglobin content (specific gravity) of the erythrocytes, but to a very slight extent as compared to the effect of the state of aggregation of the corpuscles. Clinically significant increases in rate are due almost entirely to clumping of the corpuscles into larger aggregates than usual. This tendency to clumping shows a high correlation with changes in the plasma protein content. Increase in globulin and particularly in fibrinogen or decreased albumin tend to cause an increase in clumping and, therefore, in sedimentation rate. Increase in albumin has an opposite effect.

A Normals³—A sedimentation of not over 5 mm in the first 15 minutes, and not over 15 mm in the 45 minute period is the strict normal. Anything over 30 mm in the 45 minute period may be regarded as definitely pathologic. The rates tend to be higher in women by 3 to 4 mm in 45 minutes than in men and to be higher during menstruation than at other times, but the lines of demarcation have been placed sufficiently high to include these physiologic variations in the normal values. Rates in the newborn are low, averaging 0.5 mm in 15 minutes and 2.0 mm in 45 minutes. The range which includes 95 per cent of results is less than 5.0 mm in 45 minutes based on a study of 200 children, about 10 boys and 10 girls being studied on each of the first 10 days of life. In children the rate is the same as in adults.

B Interpretation⁴—The interpretations given are based on experience in the author's laboratory with more than 100,000 determinations by the method recommended. As is true with fever and leukocytosis,

¹ Greisheimer Esther M. Treloar A. E. and Ryan Mary. The Inter Relation of Cutler Linzenmeier and Westergren Sedimentation Tests. *Am J Med Sci* 187 213 (Feb) 1934.

² Gordon M. B. and Cohn D. J. The Effect of External Temperature on the Sedimentation Rate of the Red Blood Corpuscles. *Am J Med Sci* 176 211 (Aug) 1928.

³ Osgood E. E. Normal Hematologic Standards. *Arch Int Med* 56 849-863 (Nov) 1935.

⁴ Haskins H. D. Trotman F. E. Osgood, E. E. and Mathieu A. A Rapid Method for Determination of the Sedimentation Rate of the Red Cells with Results in Health and Disease. *J Lab and Clin Med* 16 487-494 (Feb) 1931.

Mathieu, A. Trotman, F. E. Haskins H. D., Osgood E. E. and Albert J. The Sedimentation Rate in Gynecology and Obstetrics. *Am J Obst and Gynec* 21 197-204 (Feb) 1931.

an increased sedimentation rate should not be considered specific for any one condition. The chief value of the method is in calling attention to evidence of organic disease in a person who has been examined and thought to have only a functional disturbance. It is also of value in following the course of an individual patient, an increase in rate indicating progression or the development of a complication, and a decrease in rate indicating improvement or development of diffuse liver damage. The method recommended has all of the advantages of the graphic methods but is much simpler to do and record. The three points, 0 at 0 time, the reading at 15 minutes, and the reading at 45 minutes indicate the type of curve which a graph would show. If the 15 minute reading is one-third or less of the 45 minute reading, the curve is a straight line, and if the 45 minute value is within normal limits it corresponds to the horizontal line as interpreted in graphic methods.¹ If the 45 minute reading is greater than normal it corresponds to the diagonal line in the graphic methods. If the 15 minute reading is more than one third of the 45 minute reading and less than two thirds of the 45 minute reading it corresponds to the diagonal curve in the graphic methods. If the 15 minute reading is more than two-thirds of the 45 minute reading it corresponds to the vertical curve in the graphic methods and indicates extremely rapid sedimentation.

Some authors² recommend correcting sedimentation rates for anemia or decreased red cell volume. Such a correction,³ however, makes a relatively simple procedure complicated and, in my opinion, is more apt to be misleading than helpful since often corrected rates will give ridiculous results of less than 0 sedimentation and many patients with anemia, especially pernicious anemia, have normal sedimentation rates unless the illness is complicated by other disease. It seems more practical to interpret sedimentation rates of less than 50 mm in 45 minutes with caution in persons with anemia.

The causes of increased sedimentation rate are very numerous and include practically all conditions in which there is extensive inflammation, toxemia, or cell destruction, as well as pregnancy. The most important causes are

1. **Pregnancy⁴ and the Puerperium**—The rate has begun to increase by the second month, although many values still fall within

¹ Cutler J. The Graphic Presentation of the Blood Sedimentation Test. A Study in Pulmonary Tuberculosis. *Am J Med Sci* 171: 882-901 (June) 1926.

² Hambleton A. and Christianson R. A. A Simplified Method of Correcting the Sedimentation Rate for the Effect of Cell Volume. *J Lab & Clin Med* 23: 860-864 (May) 1938.

³ Cutler J. W. Iark I. R. and Herr B. S. The Influence of Anemia on Blood Sedimentation. *Am J Med Sci* 195: 734-751 (June) 1938.

⁴ This has been used as a test for pregnancy but the increase in rate occurs in too many other conditions and is too inconstant in the early months to be very reliable.

normal limits Rates as high as 30 mm in the 45 minute period may occur In the third and fourth months, the average is about 30 mm and rates above 45 mm in 45 minutes or within normal limits are unusual In the fifth and sixth months the 45 minute reading averages about 45 mm and rates up to 80 mm are not unusual In the seventh month, the average is about 50 mm and in the eighth and ninth months about 60 mm, while results under 30 mm or over 100 mm are unusual In nearly all cases more than two thirds of the sedimentation occurs after the 15 minute reading (aggregation occurs relatively slowly) An occasional normal reading may be found at any time during pregnancy

During the first five days of the puerperium, a further increase occurs, averaging about 80 mm in the 45 minutes and ranging from 40 to 120 mm There is a tendency for the 15 minute reading to be more than one third of the 45 minute reading In the next five days, the rate drops rapidly and after that more slowly, averaging about 20 mm in the second month post partum and reaching normal values by the end of this month in uncomplicated cases

2 **Infectious Diseases**—The rate is increased in all of the acute infectious diseases and in the active stages of the chronic infectious diseases The rate increases as a rule even when the leukocyte count is atypical for the condition The degree of increase of rate is greatest in pneumonia (all types, often over 100 mm in 45 minutes), but the degree of increase is so variable that it has almost no differential diagnostic value A normal rate is sufficiently rare to constitute an indication for the reconsideration of the diagnosis even though the leukocyte count may be increased The rate tends to remain elevated longer than the temperature and leukocyte count and may prove a more valuable criterion of the progress in the convalescent period In tuberculosis and syphilis the rate tends to be increased during the active stages and to decrease or be normal in latent or healed phases, but as coryza, sinusitis, and other frequently overlooked infections may increase the rate, unexpected results are frequent Miliary tuberculosis is usually associated with a more rapid rate than the leukocyte count and clinical picture would lead one to expect Nephrosis tends to give a more rapid rate (due to the high fibrinogen and low albumin) than other evidences of inflammation would suggest The rate is rapid in active rheumatoid arthritis and is normal in hypertrophic arthritis

3 **Accumulations of Pus**—The increase in rate seems to depend more on the area of absorbing surface exposed to the pus than on the virulence of the infection Increase in rate is slight or absent in

uncomplicated acute appendicitis, but constant and marked in acute salpingitis, which often has differential diagnostic value. Pleurisy, empyema, and abscesses seldom fail to cause a great increase in rate. For some unexplained reason, in peritonitis the rate is sometimes normal or even decreased, but a rapid rate is more common. As in infectious diseases the course in repeated rate determinations tends to decrease more slowly than the temperature and leukocyte count. They are thus of some prognostic value and are used by many as a guide for the time of operation in salpingitis.

4 Malignant or Necrotic Tumors—The sedimentation rate is increased at some time in the course of most malignant tumors, but this occurs late as a rule and may be absent. It is probably related to tissue destruction, necrosis of the tumor mass or secondary infection. Benign tumors do not increase the rate unless undergoing necrosis or ulceration. Peptic ulcers if uncomplicated do not increase the rate. A low rate may, therefore, be used as a point in the differential diagnosis of these conditions from malignant tumors and an unexplained increase in rate, especially if the leukocyte count is normal, should lead one to think of malignant tumors.

5 Internal Hemorrhage—Increase in rate (probably due to the associated tissue lesions) occurs in fractures, ruptured ectopic pregnancy, cerebral hemorrhage and in the lacerations of muscles or ligaments commonly called a sprain. In unruptured ectopic pregnancy, the rate is usually not increased which may aid in differentiating it from acute salpingitis.

6 Diseases of the Blood and Blood Forming Organs—The rate is usually increased in anemias and leukemias but relatively slightly in proportion to the decrease in red cells or increase in leukocytes. Rapid rates associated with a low red cell count must be interpreted with caution. A decreased rate is the rule in polycythemia.

7 Extensive Liver Disease—This may lead to a decreased rate due to decreased blood fibrinogen or to no sedimentation at all. On the other hand the decreased albumin with reversal of albumin globulin ratio in liver disease may lead to an increased sedimentation rate so that either normal, low or high rates may occur but a very low rate or no sedimentation at all should lead one to think either of severe diffuse liver disease or polycythemia rubra vera.

8 Miscellaneous Conditions—The sedimentation rate begins to increase a day or so following a coronary occlusion and is most rapid on the fifth to the eleventh days, returning to normal within three weeks to a month. In allergic diseases or heart disease not associated

with active infection, the sedimentation rate is normal. Operative procedures may cause a temporary increase in rate which returns to normal as the wound heals.

In general a sedimentation rate of 15 to 30 mm in 45 minutes should be regarded as a slight increase, of 30 to 50 mm as a moderate increase, of 50 to 100 mm as a severe increase, and over 100 mm as an extreme increase. It is also of some significance if more than one third of the sedimentation occurs in the first 15 minutes, since usually distinctly less than one third of the 45 minute sedimentation has occurred in this time. The sedimentation rate is a supplement to, but not a substitute for, repeated leukocyte counts.

IV BACTERIOLOGIC METHODS

It is not intended that this book should substitute for a textbook of bacteriology but it is desirable to review in connection with this chapter on infectious diseases the chief indications for bacteriologic studies and the common sources of error in interpreting the results of such studies.

A Indications—Smears and cultures are indicated whenever evidence of infection is obtained. This is now doubly important since specific antiserums and drugs such as sulfanilamide and related compounds which are effective against certain organisms are available. The presence of accessible pus, whether in the nares, sinuses, middle ear, sputum, pleural cavity, abdomen, hollow viscera, spinal fluid, or in urine obtained by catheter, or confined in an abscess, or draining from any sinus or orifice, constitutes an indication for smears for staining with methylthionine chloride (methylene blue), Gram or Ziehl-Neelsen stains, cultures on suitable media, or animal inoculation to identify the causative organism. The common mistake is to forget to take the culture at the first opportunity, that is until after the abscess is opened and drained and secondary infection has occurred. Blood cultures are indicated whenever typhoid fever, pneumonia, undulant fever, subacute bacterial endocarditis, puerperal sepsis or a remittent fever suggests blood stream infection. The pathogenic organisms for which culture is desired should be indicated so that suitable media for the growth of the particular organism or organisms may be selected.

B Interpretation—The presence of an organism of typical morphology in the stained smear means that and nothing more so that clinical evidence of the disease or proof by culture or animal inoculation of the identity of the organism is essential before the nature of a disease is established. For example, Gram negative intracellular diplococci in a urethral smear will, in the majority of instances, prove to be gonococci and organisms of the same morphology in a smear from centrifugated

spinal fluid will usually prove to be meningococci, but gonococcus meningitis occurs and Gram negative organisms resembling the gonococcus may be present in urethral or cervical smears. Acid fast organisms resembling the tubercle bacillus may be found in urine. In stool and throat cultures many organisms are always present and the actual organism responsible for the disease may not grow on the media selected. Cultures of foul smelling pus often prove sterile or show growth of an organism that is a secondary invader, whereas smears stained properly for identification of Vincent's organism or anaerobic cultures might reveal the true diagnosis. Taking smears alone may fail to reveal organisms that are less numerous than 500,000 per cc., and taking cultures alone may fail to reveal organisms easily demonstrable by direct smears which will not grow on the media used.

V AGGLUTINATION AND COMPLEMENT FIXATION TESTS¹

A Indications—Serum for these tests should be sent to the laboratory whenever syphilis, infectious mononucleosis, typhoid fever, undulant fever² or typhus are considered in the differential diagnosis.

B Interpretation—In the majority of conditions the tests are positive only after the disease has been present for a week or more. In some patients with these diseases the tests may never become positive. Positive tests may persist long after the disease process has become latent or the patient has recovered. Positive tests for many of the diseases may be present for some time after vaccination for prevention of the particular disease. Furthermore, there is cross agglutination between a number of related organisms and some of the tests depend on use of a nonspecific antigen, so that all that can be concluded from a single positive test is that the patient has circulating in the blood or present in the fluid tested antibodies which will react with the antigen used. If the titer rapidly increases it is strong evidence that the immunizing process is continuing and that actual infection is present but cross agglutination or agglutination due to related antigens must be excluded by the history and agglutinin absorption tests.

The interpretation of serologic tests for syphilis and of the Paul and Bunnell test requires further comment.

1 Serologic Tests for Syphilis³—Since this book is defined as a text for medical students and for practicing physicians the technic

¹ Standard textbooks of serology and immunology should be consulted.

² Evans Alice C Robinson F H and Baumgartner Leona Studies on Chronic Brucellosis IV An Evaluation of the Diagnostic Laboratory Tests Pub Health Rep 53 1507-1525 (Aug 26) 1938

³ Cumming H S Hazen, H H Sanford A H Senear F E Simpson W M and Vonderlehr R A The Evaluation of Serodiagnostic Tests for Syphilis in the United States Report of Results Ven Dis Inform 16 189 (June) 1935 J A M A 104 2083 8) 1935

of these tests is not given. The reason for this is that such tests should always be performed by a trained serologist who is thoroughly familiar with the complete description of the tests as outlined by the originators.¹ The Kolmer complement fixation test and the Kahn and Kline agglutination tests are performed by a competent serologist according to the directions of the originators are very reliable if properly interpreted.

Ideally, since syphilis is often not detected by the history and physical examination, one of the agglutination tests and a complement fixation test should be performed on the blood of each patient seen whether there is any clinical evidence of syphilis or not. Performance of only the agglutination or the complement fixation test will miss only about 5 per cent of positives which would be recognized if both were done. If positive tests are obtained by one of these methods it is desirable to have the test repeated by the other method and also checked in a different laboratory to be sure that no error has occurred due to a mixup of specimens. In untreated patients without clinical evidence of syphilis only a 3 or 4 plus report should be regarded as positive and a 1 or 2 plus report should be regarded as doubtful and an indication for repeating the test at intervals. A negative test does not exclude primary, latent or most forms of tertiary syphilis nor does it constitute evidence that sufficient treatment has been given. A negative test on the blood and cerebrospinal fluid is strong evidence against a diagnosis of secondary syphilis or general paresis in an untreated patient. Negative tests are uncommon in syphilis of the liver. Positive tests occur more often in syphilis than in any other disease but an appreciable percentage of patients with leprosy and malaria will show false positive tests and nearly all patients with yaws and bejel will show positive tests for syphilis. There is much evidence, however, that yaws and bejel are only forms of syphilis. If these diseases and infectious mononucleosis are excluded a positive test properly checked nearly always means the presence of syphilitic infection even though many negative tests have been secured before or after the positive one.

2 Paul and Bunnell Test for Infectious Mononucleosis—This test is indicated whenever sore throat, associated with generalized lymph node enlargement and the presence of prolymphocytes in the

¹ Kolmer J. A. Serum Diagnosis by Complement fixation with Special Reference to Syphilis. The Principles, Technique and Applications. Pp. 583. Lea and Febiger Philadelphia 1928.

Kahn R. L. The Kahn Test. A Practical Guide. Pp. 201. The Williams and Wilkins Co. Baltimore 1928.

Kline B. S. Microscopic Slide Precipitation Tests for the Diagnosis and Exclusion of Syphilis. Pp. 99. Williams and Wilkins Baltimore 1932.

blood leads one to consider the diagnosis of infectious mononucleosis¹. It is also indicated in patients with abdominal pain simulating appendicitis if the leukocyte count fails to show a neutrophilia.

Sheep cells are not agglutinated by the serum of normal persons in dilutions greater than 1 to 16. The presence of agglutination in a dilution greater than 1 to 32 occurs only in infectious mononucleosis or in serum disease. In infectious mononucleosis the agglutination is usually present in dilutions of 1 to 64 to 1 to 5000. In acute lymphocytic leukemia, the disease most likely to be confused with infectious mononucleosis, the heterophile antibodies may be entirely absent or agglutination may occur in a dilution of only 1 to 4. Davidsohn has developed absorption tests which will differentiate agglutinins present in infectious mononucleosis from the heterophile antibodies present in serum disease, but the history is usually adequate to make this diagnosis.

VI QUANTITATIVE DETERMINATION OF SULFANILAMIDE OR SULFAPYRIDINE

Sulfanilamide or sulfapyridine determinations are indicated in the accurate control of therapy with these drugs. The quantity of either of these drugs should not be allowed to fall below 1 mg. per 100 cc. in the blood at any time during the 24 hours, and they are most effective if the concentration is between 5 and 15 mg. per 100 cc. Toxic symptoms are likely to occur if the blood level exceeds 15 mg. per 100 cc. In the treatment of infections of the urinary tract the level should be between 50 and 100 mg. per 100 cc. of urine.

Other laboratory tests in infectious disease are given under the names of the particular diseases in the Index by Diseases.

VII THE DIFFERENTIAL DIAGNOSIS OF LEUKEMIAS²

Leukemia should be considered in the differential diagnosis whenever enlargement of the spleen and lymph nodes, bleeding into the skin or

¹ Bernstein A. Antibody Responses in Infectious Mononucleosis. *J Clin Invest* 13: 419-435 (May) 1934.

² Davidsohn I. Test for Infectious Mononucleosis. *Am J Clin Path* 8: 56-60 (Mar) 1938.

³ Forkner C E. Leukemia and Allied Disorders. Pp 333. The Macmillan Company, New York 1938.

Kracke R R and Garver Hortense. Diagnosis of Leukemic States. *J A M A*, 104: 697-702 (Mar 2) 1935.

Rosenthal N and Harris W. Leukemia. *J A M A* 104: 702-706 (Mar 2) 1935.
Riddle M C and Sturgis C C. Basal Metabolism in Chronic Myelogenous Leukemia. *Arch Int Med* 39: 255-274 (Feb) 1927.

Krantz C I and Riddle M C. The Basal Metabolism in Chronic Lymphatic Leukemia. *Am J Med Sc* 175: 229-242 (Feb) 1928.

Baldridge C W, and Fowler W M. Aleukemic Myelosis. *Arch Int Med* 52: 852-876 (Dec.) 1933.

from the mucous membranes, sore throat, stomatitis, or normocytic anemia with either leukopenia or leukocytosis are found. Although the etiology of leukemias is not definitely established it is probable that they are malignant tumors of one of the cells in the series corresponding to the type of leukemia. The more immature the cell which has undergone malignant changes, the more acute is the leukemia. The fundamental pathology is in the marrow, lymph nodes and spleen. The presence or absence of a leukemic blood picture has little or no influence on the clinical course or prognosis. Leukemias may be classified as to type according to whether the cells belong to the granulocyte (myeloid), lymphocyte, monocyte, plasmacyte, or thrombocyte series. The granulocytic (myelogenous) leukemias may be further classified into the common granulocytic (myelogenous) leukemia in which neutrophils, eosinophils and basophils are all affected and which is probably a malignant tumor of the progranulocyte A (promyelocyte II), and into neutrophilic, eosinophilic or basophilic leukemias which probably represent malignant tumors of the corresponding progranulocyte S (promyelocyte I). Granulocytic (myelogenous) and lymphocytic leukemias are common, monocytic leukemia is somewhat less frequent but more common than is generally recognized, and plasmacytic,¹ eosinophilic² and basophilic leukemias are rare.

Leukemias are classified as leukemic if the total leukocyte count is over 15,000 in the blood and over 10 per cent of the cells in the blood are immature, or in the case of chronic lymphocytic leukemia, over 70 per cent of the cells are lymphocytes. They are classified as subleukemic if the total leukocyte count is 15,000 or less and 0.1 to 10 per cent of the cells in the blood are immature cells of the involved series. They are classified as aleukemic if the total count is under 15,000 and less than 0.1 per cent of the cells present in the blood are immature cells of the involved series. In all patients with leukemia it is desirable to survey a large number of cells at a magnification of 200 times as described on page 482, looking for cells larger than the neutrophil lobocytes (polymorphonuclears) and examining these under higher magnification. More information may be derived from such a study than from the differential count. If the total leukocyte count is less than 6,000 it is desirable to centrifugate the blood and study the buffy coat. In all types of leukemia the nucleoli of the cells involved tend to be larger in

¹ Osgood E. E. and Hunter W. C. Plasma Cell Leukemia. *Fol Haematol* 52: 369-383, 1934.

² Hay J. and Evans W. H. Acute Eosinophilic Leukemia and Eosinophilic Erythro leukemia. *Quart J Med*, 22: 167-186 (January) 1929.

Forkner, C. E. Teng, C. T. Chu, Y. C. and Cochran, W. Eosinophilocytic or Eosinophilic Myelogenous Leukemia. *Chinese M J* 51: 609-618 (May) 1937.

proportion to the area of the nucleus than in the cells of the same type as seen in normal marrow. This has been found¹ true of carcinoma cells also. Stomatitis is likely to occur if the neutrophils are less than 10 per cent of the leukocytes in the blood.

Leukemias are classified as acute, subacute or chronic according to the clinical course and the predominant stage of the affected cell series present in the marrow. Since the characteristics of the different types of leukemias differ little, only variations from the typical course will be given.

A Acute Leukemias—In acute leukemias enlargement of the lymph nodes and spleen is frequent but may be absent. Petechiae in the skin and bleeding from the mucous membranes are common. Stomatitis or sore throat very often develops. Fever is nearly always present. Normocytic anemia of the myelophthisic type develops rapidly, the total leukocyte count may be anything between 1,000 and 1,000,000 per c. mm., but counts under 100,000 are most common. The blast or stem cell stage usually accounts for more than 10 per cent of the cells in the marrow and in the leukemic cases in the blood. Few cells in the marrow are more mature than the pro stage. The basal metabolism is elevated. No known therapy will appreciably prolong life which is usually a matter of only a few weeks from the onset and nearly always less than 6 months. X ray therapy usually makes the patient more uncomfortable. Sodium perborate and hydrogen peroxide treatment of the mouth and gums may prevent development of stomatitis or sore throat. Most of the acute leukemias were once thought to be of the lymphocytic type since progranulocytes (promyelocytes) may be indistinguishable from prolymphocytes in the Wright's stain. Since the use of the peroxidase stain it has been found that acute granulocytic (myelogenous) leukemias are more common than acute lymphocytic leukemias. Acute monocytic leukemias² are relatively common and differ from the other acute leukemias chiefly in a greater tendency to swelling of the gums and in the development of nodular skin lesions. The identification is made by finding promonocytes and monoblasts in the blood or increased in the marrow.

B Subacute Leukemias—In these, the clinical picture is similar to that described for acute leukemias but the tendency to hemorrhage

¹ MacCarty W. C. Identification of the Cancer Cell. J. A. M. A. 107: 844-845 (Sept 12) 1936.

² Osgood F. E. Monocytic Leukemia. Report of Six Cases and Review of 127 Cases. Arch. Int. Med. 59: 931-937 (June) 1937.

Dameshek W. Acute Monocytic (Histiocytic) Leukemia. Review of the Literature and Case Reports. Arch. Int. Med. 46: 718-740 (Oct.) 1933.

Clough P. W. Monocytic Leukemia. Bull. Johns Hopkins Hosp. 51: 148-177 (Sept) 1937.

and stomatitis develops later and the clinical course is usually 6 months to a year. The predominant cell in the marrow is the pro stage and, as in acute leukemias, more mature cells are relatively scarce. Fever is usually absent in the first few months but develops later. Therapy has little influence on the course except for the value of sodium perborate and hydrogen peroxide in controlling the mouth infection. X-ray therapy is occasionally desirable to relieve pressure symptoms from enlargement of the spleen or lymph nodes.

C Chronic Leukemias—In chronic lymphocytic leukemia the lymphocyte is the cell increased in the marrow and prolymphocytes and lymphoblasts are scarce or absent in the blood. In all other types of chronic leukemias the diagnosis is based largely on finding an increase in the pro stage in the blood or marrow but the more mature stages are also present. Leukocyte counts over 100,000 in the blood are more common in chronic leukemias than in acute or subacute leukemias. Chronic aleukemic or subleukemic leukemias also occur. Enlargement of lymph nodes or spleen may reach a greater degree and is less often absent than in the acute or subacute forms. Hemorrhage, stomatitis and fever are less common than in the acute or subacute forms. The duration of life from the onset of symptoms varies from 1 to 16 years but in the majority of instances is between 2 and 6 years. These patients are much relieved by deep X-ray therapy if enlargement of lymph nodes or spleen is causing pressure symptoms and may have months or years of useful and comfortable life. Leukopenia not due to X-ray therapy is not a contraindication to deep therapy nor is a very high leukocyte count an indication for deep therapy. As a rule the longer deep X-ray therapy can be deferred the better.

D Leukemoid Blood Pictures¹—A leukocyte count in the blood above 200,000 occurs only in leukemias but counts over 100,000 have been reported in pertussis, especially if complicated by pneumonia. The clinical picture differentiates this from chronic lymphocytic leukemia.

Counts of 50,000 to 100,000 may occur after large acute hemorrhage and, occasionally, in pneumonia or infection with the pyogenic organisms. In hemorrhage the history gives the diagnosis. In the infections eosinophils and basophils are usually absent from the blood and granulocytes (promyelocytes) rarely constitute more than 2 per cent.

¹ Krumbhaar, E. B. Leukemoid Blood Pictures in Various Clinical Conditions. *Am J Med Sc* 172: 519-532 (Oct) 1926.

of the cells present, whereas in chronic granulocytic (myelogenous) leukemia, eosinophils and basophils are usually present and the pro granulocytes (promyelocytes) constitute a higher percentage of the cells present

In patients with counts between 6 000 and 50,000, smallpox, acute osteomyelitis, Hodgkin's disease, lymphosarcoma, tumors involving the bone marrow, severe infections, polycythemia rubra vera, and acute hemorrhage have to be differentiated from chronic granulocytic (myelogenous) leukemia. The history, physical findings, bacteriologic studies, and often examination of a lymph node as well as examination of sternal marrow may be necessary to make the diagnosis with certainty. Multiple bee stings may result in a blood picture simulating chronic granulocytic (myelogenous) leukemia.

Miliary tuberculosis¹ is associated with the blood picture of subacute or chronic granulocytic leukemia too frequently to be coincidence. Some believe this leukemoid blood picture to be due to involvement of the marrow by the tuberculous process. Others, with whom I agree, believe that the leukemia is primary, softening quiescent tuberculous lymph nodes and allowing the bacilli to enter the blood stream.

The aleukemic leukemias and some of the subleukemic leukemias in which leukopenia, normocytic anemia and thrombopenia occur may be difficult or impossible to differentiate from aplastic anemia without examining the sternal marrow. The presence of large numbers of immature cells in the marrow easily differentiates leukemia from aplastic anemia in which there are few or no nucleated cells and these chiefly lymphocytes. The total nucleated cell count in the marrow of a leukemic patient is usually over 25 000 per c mm and often over 70 000, whereas in patients with aplastic anemia the nucleated cell count in the marrow is less than 6,000 and often less than 1,000 per c mm. The presence of normal or increased reticulocytes in the blood or marrow favors a diagnosis of leukemia rather than aplastic anemia. The group of splenomegalic anemias is differentiated by the absence of enlargement of the lymph nodes and by the normal sternal marrow picture. The color, volume and saturation indexes and response to antipernicious anemia principle will usually differentiate pernicious anemia, but in rare instances patients with aleukemic or subleukemic leukemias will develop a macrocytic anemia. This does not respond to liver extract, however. The differentiation of leukopenic, aleukemic or subleukemic leukemias from agranulocytosis has been discussed. The differentiation of sub

¹ Jaffé H. H. Tuberculosis and Leukemia. *Am Rev Tuberc* 2, 32 (Jan) 1933

CHAPTER VIII

DISORDERS OF THE HEMOSTATIC MECHANISM WITH ESPECIAL REFERENCE TO THE HEMORRHAGIC DISEASES

I PHYSIOLOGY AND BIOCHEMISTRY OF HEMOSTASIS¹

Much is known in this field but a great deal of speculation about the facts has been done so the known facts will be given first and the theories later

A Factors Which Prevent Coagulation—It is known that the addition of suitable concentrations of soluble oxalates, citrates or fluorides prevents coagulation. Oxalates precipitate calcium and citrates and fluorides form compounds from which calcium is not ionized. Rapid cooling of the blood or collecting the blood in paraffined or oiled containers which have no wettable surface prevents or delays coagulation. Heparin, a substance related to the carbohydrates and derived from liver or muscle tissue, will prevent coagulation for a time when added to blood in sufficient concentration. Hirudin, a substance secreted by leeches, has a similar effect as do certain snake venoms. High concentrations of neutral salts added to blood also prevent coagulation. Intravenous injection of peptone but not addition of peptone to blood in vitro prevents coagulation.

B Factors Which Favor Coagulation.—Intravascular coagulation is favored by slowing of the blood current and by roughness or a break in continuity of the vessel walls. Prompt intravascular clotting may occur if cephalin, tissue juice or ground up platelets are injected. If platelets or tissue juice are not present a clot forms but fails to retract. Retraction is normal if cephalin is added to such plasma. Transfusion of whole or citrated blood supplies the substances necessary for coagulation in hemophilia, thrombopenic purpura, or the hemorrhagic syndrome associated with complete obstructive jaundice. In vitro clotting is hastened by finely divided material with a wettable surface. The larger the wettable surface in proportion to the amount of blood exposed the more rapid is the clotting. Administration of vitamin K with bile salts reduces the bleeding tendency in jaundice. Calcium administration does not reduce the bleeding tendency in any known hemorrhagic disease and a hemorrhagic diathesis is not associated with tetany.

C The Mechanism of Coagulation—Fibrinogen interacts with thrombin to form the clot. Fibrinogen is the protein precipitated by lower concentrations of neutral salts than are necessary for precipitation of globulin and is normally present in a concentration of 0.2 to 0.4 per cent in blood plasma. Thrombin,

¹ Best C. H. and Taylor N. B. *The Physiological Basis of Medical Practice*. Pp 140-155. William Wood and Company Baltimore 1937.

Howell W. H. *Theories of Blood Coagulation*. *Physiol Rev* 15 435-470 (July) 1935.

Eagle H. *Recent Advances in the Blood Coagulation Problem*. *Medicine* 16 95-138 (May) 1937.

or thrombase, is formed when a protein compound known as prothrombin, or prothrombase, interacts with calcium ions in the presence of cephalin, tissue juice or disintegrating platelets. Calcium ions apparently are not necessary after thrombin has been formed. It is thought that the thromboplastic substance derived from tissues, platelets and cephalin, sometimes called thrombokunase, both inactivates heparin and aids in converting prothrombase or prothrombin to thrombase or thrombin. Mellanby¹ has adduced much evidence to support the belief that prothrombin and thrombin are enzymes, hence the terms prothrombase and thrombase. Vitamin K is apparently necessary for the formation of prothrombin by the liver. A congenital deficiency of fibrinogen leads to hemorrhage. In the diseases associated with decreased platelets and probably also with decreased tissue cephalin spontaneous bleeding occurs. The deficiency in hemophilia is not understood but slight trauma is necessary to start bleeding and addition of normal plasma or platelets produces prompt coagulation.

D Vascular Permeability—Bleeding may occur in the presence of normal coagulation and clot retraction if there is loss of continuity of the vessel wall or without such loss of continuity if the vessel walls are congenitally fragile or become damaged by anoxemia or toxins. The vessel walls appear to be abnormally permeable to blood in all the conditions associated with low platelet counts and in the disease scurvy. It is not yet known whether the altered permeability in scurvy is due to vitamin C itself or to a substance called vitamin P² or citrin which is found closely associated with vitamin C in foods.

E The Role of the Spleen—Removal of the spleen in some cases of purpura hemorrhagica results in the prompt cessation of hemorrhage even though the platelet count remains low. Recently, extracts of the spleen³ have been produced which, on injection, result in thrombopenia and a hemorrhagic tendency. This evidence suggests that the spleen has a regulatory influence on the production of some factor, probably cephalin, necessary for good hemostasis. Specific antiplatelet serums have a similar action to splenic extract.

II TESTS OF VALUE IN HEMORRHAGIC DISEASES

A Bleeding Time—This is indicated as a routine pre operative test and in any patient in whom spontaneous hemorrhages into the skin from the mucous membranes or from slight trauma indicate the possibility of a hemorrhagic disease.

1 **Normal**—The normal is 3 minutes or less.

2 **Interpretation**—The bleeding time is markedly prolonged in any condition in which the platelet count is low therefore, in all the conditions given on page 271 as causes for a thrombopenia. It is also prolonged in hemorrhagic disease of the newborn and in some patients with

¹ Mellanby J. Heparin and Blood Coagulation. Proc Roy Soc 116 1-9 (Sept 1) 1934.

² Jersild T. Therapeutic Effect of Vitamin P in Schonlein Purpura. Lancet 1 1445-1447 (June 25) 1938.

³ Troland C L and Lee F C. Thrombocytopen A Substance in the Extract from the Spleen of Patients with Idiopathic Thrombocytopenic Purpura that Reduces the Number of Blood Platelets. J A M A 111 221-226 (July 16) 1938.

complete or partial obstructive jaundice. The platelet count must be less than 100,000 before the bleeding time is much prolonged in the thrombopenic group, but the platelet count is so much less accurate a determination that a bleeding time should be determined on any patient on whom a platelet count is indicated. A platelet count is not necessary unless there is clinical evidence suggesting a hemorrhagic tendency or the bleeding time is found to be prolonged.

In hemophilia the bleeding time is normal.

B The Coagulation Time—This is usually determined as a routine before any operation, and in all cases in which a hemorrhagic disease is suspected. It is of practically no value unless determined by the Lee and White method on blood obtained from the vein since even hemophiliacs have normal clotting if other methods are used, and in most other hemorrhagic diseases the coagulation time is normal. Hemophiliacs usually know they have the disease. The bleeding time is far superior as a pre operative test for a hemorrhagic tendency.

1 **Normals**—These vary with the method used (see page 503)

2 **The causes of prolonged clotting time are**

(a) *Hemophilia*—This gives the most marked delay. It may require more than an hour for clotting to occur.

(b) *Hemorrhagic Diseases of the Newborn*

C Clot Retraction Time—This should be tested whenever the coagulation time is determined and in all cases of suspected hemorrhagic disease, particularly if the bleeding time is prolonged or the platelet count is low.

1 **Normals**—Retraction of the clot from the wall with separation of serum begins in one hour and is complete in 18 to 24 hours.

2 **Interpretation**—Retraction is normal in hemophilia but is markedly delayed or fails to occur in all conditions listed below as associated with a low platelet count and in hyperproteinemia the causes of which have been given on page 40.

D The Capillary Resistance Test of Rumpel-Leede—This should be done in all persons showing a hemorrhagic tendency, who have been on a greatly restricted diet, are jaundiced or are thought to have scurvy or vitamin K deficiency and is of some diagnostic value when scarlet fever is considered as a possibility.

1 **Interpretation**—Normally no petechiae or ecchymoses appear. In the following conditions which are associated with a decrease in capillary resistance or increased permeability, petechiae or ecchymoses may appear distal to the cuff or at its distal edge in a much shorter time.

(a) *Idiopathic Purpura Hemorrhagica*

- (b) *The Group of Symptomatic Purpura Hemorrhagica* (page 274)
- (c) *Scurvy*¹
- (d) *Scarlet Fever*
- (e) *In Some Patients with Vitamin K Deficiency*

Special methods of determining the capillary resistance or bleeding time by using a vacuum cup have been devised and are of some value in research but are not necessary in clinical practice

E Platelet Count and Morphology—The morphology of the platelets is described in the legend to Plate VII and should be reviewed at this time This is indicated in patients showing a tendency to spontaneous or prolonged bleeding and in all cases of suspected purpura hemorrhagica, aplastic anemia or leukemia A prolonged bleeding time, poor clot retraction or the presence of petechiae are specific indications for this examination

1 Normals—In adults and children the platelets range from 250,000 to 450,000 per cubic millimeter² In the newborn and during the first year of life the range is about 100,000 lower All technics of platelet counting are subject to errors of plus or minus 50 to 100 per cent so only variations of 100,000 or over from the normals are significant The hemorrhagic tendency does not usually appear until the platelets are below 60,000

2 Thrombocytosis, or high platelet count This may occur in

- (a) *Chronic Granulocytic (Myelogenous) Leukemia*
- (b) *Polycythemia Vera* (aids in differentiating it from secondary erythrocytosis)
- (c) *Chlorosis*
- (d) *Chronic diseases* associated with cachexia and malnutrition
- (e) *Some acute infections* erysipelas septicemia and acute articular rheumatism show the most marked changes The platelet count is usually greatly increased in moribund patients who show the toxic neutrophils indicating impending death

Increase in the platelet count is of very little diagnostic value

3 Thrombopenia—This is more important The causes of thrombopenia are

- (a) *Idiopathic or Symptomatic Purpura Hemorrhagica*—They may be as low as 10,000 per cubic millimeter and are rarely over 60,000
- (b) *Pernicious Anemia*—Counts between 80,000 and 200,000 are the rule, but a fall sufficient to give rise to a hemorrhagic tendency may occur

¹ Greene D Evaluation of the Capillary Resistance Test in the Diagnosis of Subclinical Scurvy J A M A 103 4 (July 7) 1934

² These values are for the method given on page 504 The values given for normal platelet counts vary with the technic used from 150,000 to over 10 million Olef I Blood Platelets An Improved Indirect Method for Their Enumeration Arch Int Med 46 585-596 (Oct) 1933

(c) *Idiopathic or Symptomatic Aplastic Anemia*—This diagnosis is probably incorrect if the platelets are over 100,000 per cubic millimeter

(d) *Acute or Chronic Lymphocytic Leukemia and Acute Granulocytic (Myelogenous) Leukemia*—A platelet count may be of some value in differentiating these from the chronic granulocytic (myelogenous) form. The low platelet count is not constant, however

(e) *At the onset of severe acute high fevers* such as pneumonia, malaria, and typhoid, the platelet count is said to be diminished

(f) Animals raised in the dark or on a diet deficient in vitamin A develop a thrombopenia, and this would probably apply to human beings, also

F Vitamins C and P—In scurvy, vascular permeability is increased but it is not yet certain whether this is due to a deficiency of vitamin C or to an associated deficiency of vitamin P. Since these deficiencies occur together and quantitative methods for vitamin C determination are available this determination is indicated whenever the capillary resistance test is positive or the history indicates possible scurvy or dietary deficiency

1 Quantitative Vitamin C Determinations¹—Several methods of estimating the vitamin C level in the body are available. The most practical is the determination of the blood level. Other methods depend on giving a standard dose of cevitamic acid and noting the urinary excretion or the time taken for the urinary excretion to reach a maximum. Less vitamin is excreted if there is a deficiency than if the body is fully saturated

(a) *Normals*—The strict normal blood plasma level is 0.8 to 1.4 mg per 100 cc

(b) *Pathologic Results*—Anything under 0.5 mg per 100 cc indicates definite vitamin C deficiency

G Vitamin K and Prothrombin Time²—Deficiency of vitamin K leads to bleeding apparently due to a deficient production of prothrombin. Since no method is available for direct chemical determina-

¹ Abt A. T. and Farmer C. J. Vitamin C Pharmacology and Therapeutics. J. A. M. A. 111: 1555-1565 (Oct. 22) 1938

Bessey O. A. Vitamin C Methods of Assay and Dietary Sources. J. A. M. A. 111: 1290-1298 (Oct. 1) 1938

Smith S. L. Human Requirements of Vitamin C. J. A. M. A. 111: 1753-1764 (Nov. 5) 1938

Wright, I. S., Lihensfeld A. and MacLenathen Elizabeth. Determination of Vitamin C Saturation. A Five Hour Test After an Intravenous Test Dose. Arch. Int. Med. 60: 264-271 (Aug.) 1937

² Butt H. R., Snell A. M. and Osterberg A. E. Further Observations on the Use of Vitamin K in the Prevention and Control of the Hemorrhagic Diathesis in Cases of Jaundice. Proc. Staff Meet. Mayo Clinic 13: 753-764 (Nov. 30) 1938

Quick A. J. The Nature of the Bleeding in Jaundice. J. A. M. A. 110: 1658-1664 (May 14) 1938

tion of vitamin K, a deficiency is judged by the alteration in the prothrombin time

1 **Normals**—More work is necessary before these can be accurately delimited. Prothrombin content below 50 per cent corresponding to a prothrombin time of over 15 seconds indicates definite deficiency.

2 **Pathologic Results**—If the prothrombin is below 20 per cent or the prothrombin time is over 25 seconds, there is immediate danger of bleeding and transfusions may be necessary to prevent it. The prothrombin time is prolonged in all conditions associated with deficiency of vitamin K. The most important of these is obstructive jaundice, but it seems probable that in hemorrhagic disease of the newborn and in persons on deficient diets such a prothrombin deficiency may develop.

III DIFFERENTIAL DIAGNOSIS OF THE HEMORRHAGIC DISEASES

These are all characterized by a tendency to spontaneous hemorrhages into the skin (petechiae, ecchymoses), into the joints or from the mucous membranes or to excessive bleeding from slight trauma.

A **Hemophilia**—This is a sex linked hereditary anomaly occurring only in males¹ and transmitted only through females resulting in a tendency to excessive hemorrhage from slight trauma but not from small puncture wounds. The hemorrhage is rarely, if ever, absolutely spontaneous but the trauma necessary to start it may be exceedingly slight. The platelet count and bleeding time are normal. The coagulation time is markedly prolonged but clot retraction is normal. The capillary resistance is not decreased. The best explanation so far offered is that it is due to an anomalous increased resistance of the platelets which do not break down until much later than is normally the case but a plasma factor may be responsible.

B **Purpura Hemorrhagica**² (essential thrombopenia or morbus maculosus Werlhofii)—This is an acute or chronic intermittent disease characterized by a marked tendency to spontaneous hemorrhages into the skin and from the mucous membranes. It may affect either sex. From a laboratory standpoint it is characterized by a marked decrease in the platelets (below 100 000 often as low as 5000 per cubic millimeter) and a resultant prolonged bleeding time, with normal or slightly delayed coagulation time but very poor clot retraction. The Rumpel Leede capillary resistance test is positive. The few platelets which are present are often large. As in hemophilia the erythrocytes, leukocytes and hemoglobin are normal or show the deviations from normal which are to be expected from the amount and kind of hemorrhage which has occurred thus differentiating it from the group of symptomatic purpura hemorrhagica cases described below. Splenectomy results in an apparently permanent cure of most cases of the idiopathic form.

Introduction of fresh platelets by three or four transfusions within 48 hours will produce a temporary cessation of hemorrhagic symptoms and thus permit the

¹ It is theoretically possible for it to occur in females (offspring of male hemophilic and female transmitter) but this practically never occurs.

² Eliason E L and Ferguson L K. Splenectomy in Purpura Hemorrhagica. Ann Surg 66 801-829 (Nov) 1932.

Rosenthal N. Thrombopenic Purpura. J A M A 112 101-106 (Jan 14) 1939.

The genotype corresponding to group O is OO, to group A is AA or AO, to group B is BB or BO, and to group AB is AB. A and B are dominant over O, consequently persons of genotype AO or BO cannot be separated from those of genotype AA or BB by agglutination tests.

It is evident (Table 14) that, knowing the group of the mother and child, it is possible to prove that a certain man could not be the father of the child, but it is never possible to prove that a particular man is the father of the child. For example, if the mother belongs to group O and the child to group A, it is evident that the father must belong to group A or group AB, and if the man accused belongs to group O or group B, his innocence would be established. In case of an interchange of

TABLE 14.—INHERITANCE OF BLOOD GROUPS
All Possible Combinations in Inheritance of Blood Groups (Bernstein)

Parents	Children
O × O	O
O × A	O, A
O × B	O, B
A × A	O, A
A × B	O, A, B, AB
B × B	O, B
O × AB	A, B
A × AB	A, B, AB
B × AB	A, B, AB
AB × AB	A, B, AB

infants, the blood groups of both sets of parents and both children should be determined and it may be found that one of the infants could not have belonged to one set of parents and could have belonged to the other pair. For example, if one set of parents were found to belong to group A and group A and the other to group B and group O, and one child belonged to group B and the other to group A, it is evident that the group A child could belong only to the first set of parents. However, if the child belonged to group O, it would be impossible to say to which set of parents it belonged. The chances of proving nonparentage have been calculated to be about 1 in 6 and this is increased to about 1 in 3 if the agglutinogens M and N are also determined. These agglutinogens are somewhat more difficult to determine than the ordinary blood groups but the interpretation of the results is very simple. Only three types of blood are possible: MN, N, and M corresponding to the genotypes MN, NN and MM. Any type of inheritance not listed in Table 15 is impossible.

The general rules covering the inheritance of the blood groups are that agglutinogens A, B, M or N cannot appear in the blood of the child unless present in the blood of one of the parents. A group O parent

TABLE 15—INHERITANCE OF AGGLUTINOGENS M AND N

Parents	Children
M × M	M
N × N	N
M × N	MN
M × MN	M, MN
N × MN	N MN
MN × MN	M N MN

cannot have an AB child and a group AB parent cannot have an O child, a group M parent cannot have a group N child, and a group N parent cannot have an M child.

ADDITIONAL REFERENCES

See references at the end of Chapter VI

CHAPTER IX

DISORDERS OF THE RESPIRATORY¹ AND CARDIOVASCULAR SYSTEMS²

I THE SPUTUM³

A Mechanism of Sputum Formation—The term sputum does not include saliva or nasopharyngeal secretions, but the impossibility of always preventing admixture with these must be kept in mind

Sputum consists of material brought up from the trachea and bronchi. The normal mucus secreted is too small in amount to be expectorated. It may, however, be increased to this extent by any irritation of the mucous membrane. Other sources of material are exudates of all types which will be mixed with mucus if inflammation of the tracheal and bronchial mucous membrane is associated and which can only be large in amount if cavitation connecting with a bronchus or dilatation of bronchi is present. Exudates due to bacterial inflammations will usually contain the responsible organism but may contain others as well.

Transudation into the alveoli and tracheobronchial tree may occur (pulmonary edema) just as well as into tissues (edema) or into body cavities (effusions) and has the same significance, namely passive congestion in the adjacent area.

Extensive accumulations of pathologic material may be present in the lungs, but will produce sputum only when there is a communication with a bronchus and when the material has a sufficiently fluid or friable consistency to be dislodged. Pathologic processes in adjacent regions including the peritoneal cavity, liver, esophagus, pleural cavity, and tracheobronchial glands, may penetrate the lungs to the bronchi. Therefore, the presence of sputum does not prove that the primary pathology is in the lungs and absence of sputum does not exclude pathology involving the lungs.

Blood may enter the bronchi and be expectorated, due to passive congestion, to ulceration, or to hemorrhagic inflammation involving the bronchi or communicating with them.

B Indications and Instructions for Collection of Sputum—
In any case in which sputum is expectorated, sputum examinations

¹ Willis H. S. *Laboratory Diagnosis and Experimental Methods in Tuberculosis* Pp. 330. Charles C. Thomas, Springfield, Illinois, 1928.

² Notwithstanding the great frequency and importance of diseases involving these systems, clinically practical laboratory aids to diagnosis are relatively few and many of these have been previously discussed. Hence a general resumé does not seem justified.

³ Wells H. G., DeWitt Lydia M. and Long E. R. *The Chemistry of Tuberculosis* Ed. 2. Pp. 481. Williams and Wilkins Company, Baltimore, 1932.

should be performed. If bronchial asthma is suspected, a Wright's stain for eosinophils is indicated in addition. In pneumonia, typing of the pneumococci is necessary if serum treatment is to be intelligently used. In most instances repeated examinations are necessary. Information of greatest value is secured by collection, measurement and examination of the total 24 hour volume. Specific directions for such collection should be given. In infants swabbing the pharynx during coughing may be necessary to obtain sputum as it is frequently swallowed. Older children may often be taught to expectorate. Coughing of any type should lead one to attempt to secure sputum for even coughing reported to be nonproductive may yield traces of material which repay examination, and failure to obtain sputum is in itself of significance.

Time spent in explaining to the patient the importance of collecting only material which is coughed up not saliva or material aspirated into the throat from the nasal cavity, and all of that material (no swallowing of sputum) as uncontaminated as possible (rinsing the mouth after meals, abstinence from chewing tobacco, etc.) is usually well repaid. Clean and if cultures are to be made sterile containers of suitable size should be provided.

C Interpretation — 1 The Volume Expectored in 24 Hours — A volume of over 100 cc per day may occur in pulmonary edema, lung abscess, bronchiectasis, pulmonary gangrene, advanced pulmonary tuberculosis or from any extrapulmonary collection of pus or fluid which connects with a bronchus such as empyema, liver abscess or subphrenic abscess. If pulmonary tuberculosis has been diagnosed a daily expectoration of over 25 cc suggests cavitation or bronchiectasis. Smaller volumes do not exclude these diagnoses. The alteration in the 24 hour volume of the sputum from day to day in acute cases and from week to week in chronic cases is of prognostic value. Increasing quantities in abscess, cavitation or bronchiectasis indicate progression, gradual decrease indicates healing and sudden decrease indicates a plugging of the ostium which is apt to be followed by a flare up of constitutional symptoms if drainage is not re-established. Cysticercosis or abscess may of course be present without communicating with a bronchus, hence little or no sputum does not exclude these possibilities.

2 The Appearance and Consistency — It may be mucoid, mucopurulent, purulent, serous, or bloody, according to the character of the inflammation producing it. The sputum of lobar pneumonia is very sticky and tenacious, that of lung abscess, bronchiectasis, and pulmonary gangrene has a tendency to separate into 3 layers if allowed to

stand It is watery and frothy in pulmonary edema and usually blood tinged

3 **The Color**—Jaundice or pyocyanous infection may give it a green color Breakdown of anthracotic tissue may cause it to appear black In lobar pneumonia the sputum is blood tinged after the first few days, later becoming rusty and finally like prune juice in color The sputum from amebic abscess of the liver which has ruptured into a bronchus has a characteristic anchovy sauce appearance

The causes of blood in the sputum in the approximate order of importance are

(a) *Pulmonary Tuberculosis* (60 to 80 per cent of all cases) —Blood may be in traces or large hemorrhages

(b) *Pneumonias of All Types*—In the streptococcic pneumonia often associated with epidemic influenza, blood is more abundant than in other pneumonias

(c) *Chronic heart disease*, especially mitral stenosis (passive congestion or infarction)

(d) *Bronchiectasis, Gangrene, or Lung Abscess*

(e) *Pulmonary Infarction* (inconstant but of confirmatory value when present)

(f) *Malignant neoplasms*, particularly carcinoma of a bronchus Hemoptysis may be due to ulceration of carcinoma of the thyroid or esophagus into a bronchus

(g) *Hypertension*—Bleeding is at times profuse The mechanism is not clear

(h) *Ulcerations of the respiratory passages, penetrating wounds of the chest, and foreign bodies in the bronchi*

(i) *Hemorrhagic diseases*

(j) *Aneurysm of the Thoracic Aorta* (slight bleeding for months, or sudden large hemorrhage)

(k) *Gassing in Warfare* (immediate and delayed) —It has been reported as long as 2 years afterward

(l) *The Rare Diseases* spirochetal bronchitis, epidemic hemoptysis (due to *Paragonimus westermani*¹), and broncho moniliasis

Pure blood unmixed with sputum occurs in all large hemorrhages and in groups (g), (i), and (j) as a rule, and often in bronchiectasis, infarction, and carcinoma

Hemoptysis should be regarded as due to pulmonary tuberculosis until proof of another cause can be established, but the frequency of hemoptysis in bronchiectasis has not been sufficiently stressed

4 **The Odor**—A very foul odor is present in pulmonary gangrene and putrid bronchitis and often in bronchiectasis and lung abscess

¹ Ova of this trematode in the sputum establish the diagnosis

A peculiar sweetish sickening odor may also be present in the latter two, but odor is far more commonly absent than textbook descriptions would lead one to believe. A foul odor may also result from necrosis of malignant tumors such as carcinoma of a bronchus, but is a late symptom more often absent than present. At least one clinician, however, has established a reputation for accuracy in the diagnosis of bronchial carcinoma, which he claims to make on the basis of the presence of a faint but characteristic odor of the sputum or exhalations.

5 Elastic Tissue—The presence of elastic fibers in sputum indicates the breaking down of lung parenchyma, and hence, is a very important diagnostic and prognostic point. It would speak for lung abscess, gangrene, active cavity formation, or ulcerating malignant tumors and does not occur in uncomplicated bronchiectasis, uncomplicated non tuberculous pneumonias, in sputum from non progressing cavities, or in material entering the bronchi from other sources than the lungs such as empyema, subphrenic or liver abscess and caseous mediastinal nodes.

6 Evidences of Bronchial Asthma—*Curschmann's spirals* and *Dittrich's plugs* strongly suggest bronchial asthma and the presence of large numbers of *eosinophils* and *Charcot Leyden crystals* are still more conclusive evidence for this diagnosis.

If eosinophils are absent from the sputum a diagnosis of bronchial asthma is probably incorrect. This is a very valuable point, for bronchial asthma is one of the diagnoses most commonly made erroneously. Further search in such cases will usually reveal a cause for left ventricular failure such as hypertension, aortic stenosis or aortic insufficiency and thus show that the shortness of breath is paroxysmal nocturnal dyspnea which is sometimes called cardiac asthma.

7 Bronchial Casts—These are rare. They consist of masses of fibrin and constitute the basis for the diagnosis of fibrinous bronchitis, a cause for which should be sought by bacteriologic studies.

8 Tubercle Bacilli¹—A search for tubercle bacilli should be a part of the routine examination of the sputum and, if tuberculosis is clinically suspected, at least ten 24 hour collections of sputum should be examined by concentration methods, before a failure to find the organism is considered significant. Then, if the organism is not found, it is justifiable to conclude only that tubercle bacilli are either not present in the sputum or are not entering it in sufficient

¹ It has been my policy to omit discussion of the bacteriologic and serologic phases of laboratory diagnosis as they are adequately covered in other courses and texts. I deviate in this instance because misinterpretation of the significance of finding or failure to find tubercle bacilli in the sputum is so common.

numbers to be detected by the method used. If clinical suspicion of tuberculosis persists, guinea pig inoculation or cultural methods¹ of identification are indicated and examination of the sputum by concentration technic should be repeated at intervals.

Failure to find tubercle bacilli by any method does not exclude tuberculosis. Tubercle bacilli are not to be expected in the sputum in miliary tuberculosis, in tuberculous pneumonia before the caseous material softens sufficiently to be expectorated, or in many cases of fibroid tuberculosis.

On the other hand, inability to find tubercle bacilli in the sputum of a patient in whom cavitation has recently developed or is progressing, as determined by roentgenographic evidence and physical signs, speaks strongly against tuberculosis as the etiologic agent. The largest numbers of tubercle bacilli are found in the sputum during the breaking down period of a caseous focus or caseous pneumonia, and they tend to be present in the sputum as long as tuberculous activity in the walls of the cavity is progressive. Tubercle bacilli may be present in the sputum in the absence of active pulmonary disease from rupture of a caseous node into a bronchus but this is relatively uncommon. The presence of tubercle bacilli does not exclude the coexistence of carcinoma, non tuberculous bronchopneumonia, or silicosis. Repeated sputum studies at intervals with notation in a roughly quantitative way of the numbers of organisms present is of value in conjunction with the clinical findings in gauging the course of the disease.

II EXUDATES, TRANSUDATES, EDEMA, SECRETIONS AND OTHER FLUID COLLECTIONS²

A Mode of Formation—The mechanism of the formation of a collection of fluid in a body cavity is the same as in the tissues. Exudates are reactions of the body to irritative injury, and vary in character according to the nature and intensity of the inciting agent. They have a limited degree of type specificity as illustrated by the differences in character of exudates due to the pyogenic organisms and those due to tubercle bacilli. Only the demonstration of the causative organism is adequate proof of their etiology, and it is possible for this to be misleading as in secondary infection of a tuberculous effusion.

Transudates in the body cavities are entirely analogous in formation and composition to edema³ in the tissues. Normally, fluid leaves the first part of the

¹ Corper H J and Cohn M L. Routine Clinical Examination for Tubercle Bacilli in Microscopic Negative Sputums by Various Culture Methods. J Lab and Clin Med 18: 515-520 (Feb) 1933.

² Wells H G. Chemical Pathology. Ed 5. Pp 363-407. W B Saunders Philadelphia 1925.

³ Peters J P and Van Slyke D D. Quantitative Clinical Chemistry. Vol I pp 653-724. Williams and Wilkins. Baltimore 1931.

Gilligan D R, Volk M C and Blumgart H L. Observations on the Chemical and Physical Relation Between Blood Serum and Body Fluids. I. The Nature of Edema.

capillaries because the capillary pressure is greater than the colloid osmotic pressure, and is reabsorbed at a corresponding rate at the venous end of the capillary where the intracapillary pressure is lower than the colloid osmotic pressure. Anything which lowers the colloid osmotic pressure, increases the extracapillary osmotic pressure or increases the intracapillary pressure tends to the production of edema. The colloid osmotic pressure is normally about 30 mm of mercury and is due chiefly to the albumin fraction since 1 gram of albumin in 100 cc of plasma exerts about 5.5 mm of mercury pressure and 1 gram of globulin exerts only about 1.4 mm of mercury pressure. Edema usually develops when the albumin fraction falls below 2.5 grams per 100 cc and the edema fluid is very low in protein content. Edema of this type occurs chiefly in nephrosis and dietary deficiency and may occur in any of the conditions listed on page 40 as causes of hypoproteinemia. Edema is nearly always generalized in this group.

An increase in permeability of the capillary walls permits protein to escape from the vessels and to raise the extracapillary pressure so that the difference between the extracapillary and intracapillary colloid osmotic pressure is reduced. This type of edema occurs in most forms of inflammation, as a reaction to histamine, many poisons including bee venom and the poisons of most stinging or biting insects, and in acute, subacute and chronic glomerular nephritis. Edema is generalized if the increased permeability is due to a circulating toxin as in nephritis but is localized when it is due to local injury as from a bee sting, a sprain or in rheumatic arthritis. The protein content of the fluid is high in this group and approaches or equals that of exudates.

Increased intracapillary pressure results from venous stasis and accounts for the edema in congestive heart failure, thrombosis of the veins and obstruction of venous flow by pressure of tumor masses. A certain amount of anoxemia is usually associated which tends to increase capillary permeability so that the protein content of the edema fluid is greater than in edema due to decreased serum proteins but not as high as in inflammatory edema. If the increased pressure is generalized, as in congestive heart failure, the edema is greatest where the pressure is greatest, namely in the most dependent portions. When the increased venous pressure is local the edema occurs only at that site.

Tissue pressure influences the distribution of generalized edema, which is greatest at sites such as the eyelids or scrotum where the tissues are loosest and tissue pressure is least.

Obstruction to lymph channels tends to produce chylous accumulations of fluid.

Cyst formation seems to be largely a matter of secretory activity of the tissue forming the wall. Another method of cyst formation is obstruction to a normal fluid pathway. Hence, the composition of cyst fluids will vary greatly.

B Indications for Examination—Any pathologic collection of fluid including abscess, draining sinus, exuding wound surface, cyst, effusion in pleura, pericardium, peritoneum, joint or bursa, bleb,

vesicle, or pustule, which is removed at operation or by puncture should be sent to the laboratory for study, and puncture for diagnostic purposes is indicated in most fluid accumulations. Puncture should not be performed on cystic tumors within the abdomen, except at laparotomy under direct vision. Hence, paracentesis abdominis should be limited to cases in which the presence of ascites is well established by the physical examination. A greatly dilated stomach containing much fluid should be excluded by passage of a stomach tube. Large cystic tumors may, in rare instances, cause doubt, in which case laparotomy is safer. Extensive adhesions from healed peritonitis are further contraindications.

On the other hand, paracentesis thoracis is a valuable diagnostic procedure in cases in which difficulty arises in differentiating thickened pleura from effusion and has little danger in competent hands.

Examination of secretions from the nose, accessory sinuses, conjunctiva, middle ear, joint, bursae, urethra, prostate, cervix or vagina is indicated whenever present in excess of the normal amount.

Examination of the semen is indicated as the first step in determining the cause of sterility.

C Interpretation¹—1. **Transudates**—These are noninflammatory collections of fluid. They are usually secondary to passive congestion, hypoproteinemia or local or general vascular injury. The fluid is clear, of a light straw color, with a specific gravity less than 1.018 and usually less than 1.015. They are further characterized by a protein content of less than 30 grams per liter, a negative Rivalta test, and a low cell count. Red cells, if present, are few in number and due to the puncture wound itself. Spontaneous clotting does not occur. After repeated tapplings, there is a tendency for eosinophilic cells to be numerous in fluids removed and for the character to approach that of an exudate. Eosinophilic cells are usually numerous in the collection of fluid associated with artificial pneumothorax. In edema fluids due entirely to hypoproteinemia the protein content is usually less than 10 grams per liter and may be less than 1 gram per liter. In edema fluids due to increased vascular permeability the protein content is usually over 20 grams per liter. In edema fluids due to increased venous pressure the protein content is usually between 15 and 25 grams per liter.

The common sites for transudates are the peritoneal cavity from cardiac failure or obstruction of the portal vein, the right pleural

¹ Foord A G, Youngberg G E and Wetmore Vera. *The Chemistry and Cytology of Serous Fluids*. J Lab and Clin Med, 14 417-423 (Feb) 1929

cavity (less often the left), the scrotum the subcutaneous tissues and the pericardium. Nephrosis and glomerular nephritis are often associated with general anasarca. The fluid in nephrosis tends to be extremely low in specific gravity and protein content, while that in nephritis is near the borderline between an exudate and a transudate in specific gravity and protein content. As the transudate fluid contains nitrogenous end products of metabolism in proportions similar to those in the blood, it is possible that removal of transudate fluid¹ in cases of renal impairment would have therapeutic value from the standpoint of relieving the load on the kidneys.

2 **Exudates** —These are collections of fluid secondary to an inflam-
matory process. They may be clear, turbid, or grossly purulent, depending on the number of cells present. Blood is not infrequently noted. Their most constant characteristics are a specific gravity of 1.018 or more, a positive Rivalta test, and a protein content of over 30 grams per liter. Spontaneous coagulation and an increased white cell count are also usually observed.

(a) *Tuberculous Effusions* —These are, as a rule, clear or slightly turbid, straw colored, and coagulate spontaneously. Much less commonly they are bloody. The predominating cells are small lymphocytes. The tubercle bacillus may occasionally be found in the stained sediment after centrifugation, and more often by guinea pig inoculation with this sediment. Inability to demonstrate the presence of tubercle bacilli by these methods does not exclude a tuberculous origin.

(b) *Effusions Due to Malignant Tumors* —These are often bloody but may be clear. The cytology varies. In some cases specific tumor cells may be detected by sectioning the centrifugated sediment.²

(c) *Exudates Due to Pyogenic Organisms* —These are usually cloudy or grossly purulent. The predominating cells are neutrophils and the offending organism can usually be demonstrated in the stained smear or by culture.

3 **Chylous and Pseudochylous Effusions** —These are characterized by their milky appearance which does not disappear on centrifugation. True chylous effusions are rare and are due to lesions of the thoracic duct (filariasis most commonly). The milky appearance is due to finely divided fat and therefore they become clear on extraction with ether. Pseudochylous effusions have the same appearance but they are not cleared by ether extraction and are not due merely to fat. Their significance is not known.

¹ Experimental work on the value of induced transudation, removal of the transudate fluid, and replacement of the protein loss in part by transfusion in animals in which uremia has been produced would seem to be a profitable line of investigation.

² Zernitsky, A. I., Jr. Examination of Fluids for Tumor Cells. Analysis of 114 Cases Checked Against Subsequent Examination of Tissue. *Am. J. Med. Sc.* 55: 492, 504 (April) 1918.

4 **Cyst Fluids** ¹—The cysts of *Taenia echinococcus* are recognized by the presence of the characteristic hooklets in the fluid removed by operation. Puncture should not be done because of the danger of dissemination or violent anaphylactic reactions if any of the cyst contents escape about the needle or through the puncture opening.

Cysts containing urine are recognized by the high urea content. The fluid usually contains over 500 mg of urea nitrogen per 100 cc, whereas other cyst fluids and puncture fluids contain urea nitrogen in quantities similar to that in the blood of the patient.

Certain ovarian cysts contain a slimy mucinous material called pseudomucin which differentiates them from Graafian follicle cysts which contain serous fluid and other cystic tumors of that region which are less apt to become malignant than are the cysts containing pseudomucin.

Dermoid cysts or teratomas contain a thick, buttery, sebaceous material usually associated with hair, teeth, or bone. Sebaceous material alone is present in sebaceous cysts.

5 **Secretions**—The presence of pus in any secretion indicates infection and the bacteriologic study of that secretion by stains and cultures for the causative organism. Eosinophilia found in the secretion from the nose suggests vasomotor rhinitis or hay fever. The variety of organisms likely to be found varies with the sources of the secretion, streptococci and staphylococci being the most common and important organisms found in the secretions of the accessory sinuses, middle ear joints, or bursae. Search for gonococci is especially important in the secretions from the conjunctiva, urethra, prostate or cervix. A conjunctival purulent secretion should also be examined for Koch Weeks bacilli, pneumococci or Morax-Axenfeld bacilli. Cholesterol crystals found in the material obtained from the auditory canal indicate the presence of cholesteatoma. The presence of *Trichomonas vaginalis* in moist cover slip preparations of vaginal secretion is characteristic of one type of leukorrhea.

6 **Semen**—(a) *Normal Values*—The volume is 3.5 to 7.0 cc, average 5.0 cc. At least 75 per cent of the spermatozoa are normal in configuration and actively motile. The count is 70 million to 200 million spermatozoa per cc, with an average of about 500 million per ejaculation. The fluid is viscid, opalescent, greyish white and contains granules which liquefy on standing.

(b) *Interpretation*—The presence of pus or blood indicates infection in the prostate or seminal vesicles. Complete absence of spermatozoa called azospermia or complete absence of motility in repeated specimens indicates absolute sterility. A volume of less than 3.5 cc, a spermatozoon count of less than 50 million per cc, less than 25 per cent of motile forms or the presence of many deformed spermatozoa indicates relative infertility. Lack of motility or absence of spermatozoa in the cervical secretions after coitus indicates an abnormality of the cervical or vaginal secretions providing the seminal fluid has been proved to be normal.

III THE VITAL CAPACITY²

The diagnostic value of this test is not yet fully established, but it appears to be of some value in prognosis and in following the course of patients with diseases of the respiratory or cardiovascular systems.

¹ Details should be sought in special works on pathology. Much is yet to be learned of the chemistry of cyst contents.

² Myers J. A. *Vital Capacity of the Lungs*. Pp. 140. Williams and Wilkins, Balt.

A Normals—There are many different standards but those based on surface area appear at present the most satisfactory. For men, 2.5 liters per square meter for women, 2.0 liters per square meter, and for children 1.9 liters per square meter of body surface are considered the average normal. Results may also be calculated from standing height. The figures per cm of standing height are 250 cc for men 200 cc for women and 155 cc for children. Athletes have vital capacities averaging about 10 per cent higher than other normal individuals. More detailed data and tables on normal values will be found in Myers' book.¹

Results are reported as per cent of the normal. About 90 per cent of the normals fall between 85 and 115 per cent of the normal standard, a few giving estimations as low as 75 per cent or as high as 125 per cent. Normal negroes average about 20 per cent lower than normal white persons.

B Pathologic Variations—Any reading below 70 per cent of the normal may be considered definitely pathologic, although many cases with respiratory or cardiac pathology give higher readings. Any reading below 40 per cent of the normal indicates marked respiratory or cardiac insufficiency. The chief value of the test seems to be in following the progress of a patient from day to day. Much work still must be done on it. Apparently any condition which encroaches on the intrapulmonary air space will decrease the vital capacity. In heart disease, engorgement of the pulmonary vessels is probably the major factor, with hydrothorax, pulmonary edema and infarction becoming more important when they occur. In pulmonary disease pneumothorax, effusions, loss of expansibility, as from adhesions or fibrosis, occlusion of airways, or replacement of air space by exudate as in pneumonia or abscess, or by new tissue as in fibrosis or tumors would all be factors. Neurologic or skeletal disturbances impairing the expansibility of the chest wall or diaphragm would decrease it, as would crowding upward of the diaphragm. Hence decreased vital capacity is far from specific but would seem to be of value as a test of functional efficiency.

IV CAPILLARY MICROSCOPY

This is of some value in the thorough study of Raynaud's disease, scleroderma, glomerulonephritis and other vascular disturbances but is as yet largely of research interest. Details should be sought in the writings of Krogh,² Brown and others.

The reader is referred to the Index by Diseases for a summary of the indications for and results of laboratory tests in the specific diseases of these systems in which such tests are important.

more 1925

Moersch H J. The Vital Capacities of One Thousand Surgical Patients. Arch Int Med 37: 128-143 (Jan) 1926

Lemon W S. Historic Review of American and English Literature on Vital Capacity. Minnesota Med 14: 1031-1041 (Dec) 1931

¹ See footnote 2 on page 283

² Krogh A. The Anatomy and Physiology of the Capillaries. Pp 422. Yale University Press 1929

Brown George E. The Skin Capillaries in Raynaud's Disease. Arch Int Med 35: 56-73 (Jan) 1925

Brown G I and Ioth Grace M. Biomicroscopy of the Surface Capillaries in Normal and Pathologic Subjects. M J Australia 1: 499-506 (April 2) 1927

See also monograph by Kylin cited at the end of Chapter II

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CHAPTER X

DISORDERS OF THE CENTRAL NERVOUS SYSTEM WITH ESPECIAL REFERENCE TO THE DIFFERENTIAL DIAGNOSIS OF COMA

I THE CEREBROSPINAL FLUID¹

A Resume of the Essential Points in the Anatomy, Physiology, and Biochemistry of Cerebrospinal Fluid Formation

The cerebrospinal fluid is formed by the choroid plexus in the ventricles of the brain by a process of dialysis² through a selectively permeable membrane. The normal volume is about 120 cc. It differs from plasma chiefly by the absence of colloids and pigments, the greater concentration of chloride and the lesser concentration of dextrose (see Table 16). Its direction of flow is from the lateral ventricles through the foramina of Munro into the third ventricle, thence through the aqueduct of Sylvius to the fourth ventricle and out through the foramina of Luschka (and Magendie³) into the subarachnoid space over the surface of the brain and cord. It was formerly thought to be reabsorbed only through the Pacchionian granulations into the venous sinuses of the dura, but Dandy⁴ has produced evidence suggesting that it may also be absorbed by the capillaries of the pia-arachnoid.

The composition of the cerebrospinal fluid is altered by any one of the following fundamental changes: by any exudative inflammation of the leptomeninges, by any alteration in the composition of the blood as regards substances to which the choroid plexus is permeable, by any change in permeability of the choroid plexus, or by any increase in permeability or any solution of continuity of the blood vessels at the surface of the brain or cord or in the normal cavities of the brain.

As the brain and spinal cord are enclosed within a non-expansile bony cavity, any increase in the total content of this cavity will tend to increase intracranial pressure. Such increase may be due to one of the following: increased volume of cerebrospinal fluid (caused by any obstruction to the normal course of flow or by increased rate of formation), transudate (edema of the brain), exudate (meningitis, abscess), or increased volume of blood (hemorrhage, obstruction to the venous outflow). With the exception of obstruction to the reabsorption of

¹ Greenfield, J. G., and Carmichael, E. A. *The Cerebrospinal Fluid in Clinical Diagnosis*. Pp. 272. MacMillan Co., New York, 1915.

Ayer, J. B. *The Analysis of Spinal Fluid Tests*. J. A. M. A., 87: 377-379 (Aug. 7) 1926.

See also references at end of this chapter.

² There is some evidence that the cerebrospinal fluid is at least in part a secretion of the cells of the choroid plexus, but for practical clinical purposes it may be regarded as a dialysate of the plasma.

³ Dandy, W. E. Where is Cerebrospinal Fluid Absorbed? J. A. M. A. 61: 2012-2014 (June 15) 1929.

the cerebrospinal fluid any change which is to increase pressure must occur rapidly, or it will be compensated by a corresponding increase in the rate of absorption. Tumors may attain considerable size without increasing the intracranial pressure if they do not obstruct the flow of this fluid, while others of small

TABLE 16—COMPARISON OF NORMAL CEREBROSPINAL FLUID AND BLOOD PLASMA*

	Cerebrospinal fluid		Plasma
	Range	Average	Average
Specific gravity	1 006 to 1 009	1 0075	1 025
Total solids†	0 83 to 1 77	1 00	8 7
Water content‡	98 23 to 99 17	99 00	91 3
Freezing point— C	-0 534 to -0 603	-0 570	-0 570
Chloride§	424 0 to 454 0	440 0	360 0
Chloride (as NaCl)‡	700 0 to 750 0	726 0	594 0
Bicarbonate§		21 0	23 0
Phosphorus‡	1 to 2 0	1 5	4 0
Lactic acid‡	10 0 to 20 0	15 0	15 0
Sodium‡	301 0 to 343 0	324 0	316 0
Potassium‡	11 0 to 15 0	13 0	19 0
Calcium‡	4 5 to 5 5	5 0	10 0
Magnesium‡	1 0 to 3 5	3 0	2 0
Total base§		155 0	162 0
Protein‡	15 0 to 45 0	28 0	7000 0
Albumin		23 0	4430 0
Globulin		5 0	2270 0
Fibrinogen			300 0
Nonprotein nitrogen‡	11 0 to 38 0	19 0	27 0
Urea‡	8 0 to 8 0	14 0	14 0
Creatinine‡	0 5 to 1 9	1 1	1 6
Amino acid‡	1 2 to 2 0	1 6	5 0
Uric acid‡	0 4 to 2 8	1 7	4 7
Cholesterol‡	0 06 to 0 22	0 14	160 0
Reducing substances‡	50 0 to 80 0	65 0	98 0
Glucose		61 0	92 0
Nonglucose		4 0	6 0

* Reproduced by permission of the authors and publishers from Merritt H H and Fremont Smith
F The Cerebrospinal Fluid P 12 W B Saunders Co Philadelphia 1937

† Grams per 100 cc

‡ Milligrams per 100 cc

§ Millimols per liter

size so placed as to cause obstruction may produce great increases in pressure. Normally, the fluid column in the spinal canal is in continuity with that in the cranial cavity, and in the horizontal position, the pressure will be the same in each. Any lesion involving only the interior of the nervous tissue and not approaching the ventricles, and any entirely extradural lesion will not influence the composition of the cerebrospinal fluid, although it may influence its pressure.

B Indications for Examination of the Cerebrospinal Fluid —

This is indicated not only in all cases of coma but also in the absence

of coma when any of the following conditions are included in the differential diagnosis: meningitis of any type, central nervous system syphilis of any type, miliary tuberculosis, cerebral hemorrhage of any type, injuries to the skull or spine, tumors or abscesses of the brain or cord, multiple sclerosis, anterior poliomyelitis, or lethargic encephalitis and in any obscure condition with symptoms or signs of central nervous system involvement. In addition, it may be of value in severe mastoiditis (to detect early evidence of sinus thrombosis, or a tendency to invade the meninges), in severe frontal, ethmoid, or sphenoid sinusitis, in osteomyelitis of the cranial bones or vertebrae (to detect early evidences of a tendency to invade the meninges), and in any case of syphilis to detect evidence of invasion of the central nervous system. It is performed for therapeutic purposes in edema of the brain such as occurs in eclampsia, from hypertension, after head injuries, and in many other conditions. Lumbar puncture in the interspace between the fourth and fifth or third and fourth lumbar spines is recommended for most purposes.

If evidence of a subarachnoid block is obtained, cisternal puncture should be done. Some recommend cisternal puncture as the usual method because it is technically easier than a lumbar puncture, but even in the most skilled hands a cisternal puncture occasionally results in death from puncture of the medulla or anomalous veins.

C Contraindications to Spinal Puncture—Many authors list a considerable number of contraindications to spinal puncture, chief of which are cerebral hemorrhage and tumors in the posterior fossa. In my experience many more mistakes occur from failure to examine the fluid than from neglect of these contraindications. The danger of increasing a cerebral hemorrhage is slight if the minimum amount of fluid necessary for study is *slowly* removed. In all cases of increased intracranial pressure, as evidenced by papilledema, a very gradual reduction in pressure is desirable with close watch for the development of respiratory symptoms, as the medulla and tonsil of the cerebellum may otherwise occasionally be forced into the foramen magnum and death result from compression of the vital centers in the medulla. Because of this danger a lumbar or cisternal puncture should never be done if a tumor of the posterior fossa is suspected. A puncture should never be made through an infected area. If high grade papill edema is present, trephining the skull and puncture of the ventricles is a safer procedure.

D Technic for Securing Cerebrospinal Fluid—1. **Lumbar Puncture**—The patient should lie on the side with the knees drawn

up and the head flexed and supported on a pillow so that the plane of the back is at right angles to the floor and the back is in maximum flexion. Locate the interspace between the fourth and fifth or third and fourth vertebral spines by palpation and mark the center by pressure with the thumb nail. Prepare this area with iodine and alcohol and drape with a sterile towel having a small hole in it or with several towels. Wearing sterile rubber gloves and using aseptic technic infiltrate the skin and subcutaneous tissues with 1 or 2 per cent procaine hydrochloride. Wait 3 or 4 minutes for the anesthetic to take effect. This wait is the most important point in making the puncture painless. Anesthesia is not necessary if the patient is in coma. Such patients and irrational patients or children should be held firmly by strong assistants. Use an 18 gauge needle of nonrusting material preferably with a two way stopcock for connection to a manometer. During introduction the needle should be held with the right hand and guided by the left hand supported against the back so that a sudden movement of the patient or give of the tissues will not result in a sudden thrust or bend of the needle. Introduce it at or near the midline at right angles to a tangent to the curve of the back at the point marked and at the correct angle so that the point will reach the midline at the proper distance for the estimated depth of the spinal canal in a patient of the subject's build. A slight give is felt as the dura is pierced. Withdraw the stylet just enough to note whether fluid appears in the lumen. If it does, attach the manometer and take pressure readings as described below. When these are completed allow the fluid to drip slowly into sterile small test tubes until 2 or 3 cc have been collected in each of 2 or 3 tubes. The last or clearest fluid should be used for the cell count and protein determination.

Some prefer smaller needles or the Greene needle since these lessen the incidence of headaches but the larger needles are necessary if pressure studies are to be accurate and to obtain fluid in acute purulent meningitis. Ambulant patients should be advised to spend the next 24 hours in the reclining position if they wish to avoid headaches. A high fluid intake if there are no contraindications will also aid in preventing such postpuncture headaches.

2 Cisternal Puncture - The patient is placed in the position described for lumbar puncture and the same type of needle is used, but ideally it should have a mark 7.5 cm from the tip and a short bevel. The region between the occiput and the spine of the atlas is shaved and prepared as described for lumbar puncture. The skin is anesthetized at a point just above the spine of the atlas in the midline and

Counts between 10 and 100 per c mm—These are most characteristic of general paresis, tabes dorsalis, vascular neurosyphilis, encephalitis, encephalomyelitis, and anterior poliomyelitis. They may occur in any of the conditions listed as giving higher counts. Counts between 10 and 100 per c mm occur in some cases of multiple sclerosis, uremia, trypanosomiasis, and epilepsy.

If a pleocytosis is found, a differential cell count on the stained centrifugated sediment, stains and cultures for bacteria, and determination of cerebrospinal fluid dextrose and chloride are indicated.

H Differential Cell Count—This is indicated on all spinal fluids showing a pleocytosis. In normal fluids the cells are all lymphocytes.

Neutrophils predominate in all of the conditions listed as giving typical counts above 500 per c mm. This is true also of the most acute cases of tuberculous meningitis and syphilitic meningitis and may occasionally occur in poliomyelitis.

Lymphocytes and neutrophils are both present with lymphocytes usually predominating in the group of conditions listed as giving a typical cell count of between 100 and 500 per c mm.

Lymphocytes predominate and are usually the only type of cell present in the group of conditions listed as giving a typical cell count between 10 and 100 per c mm. Lymphocytes are almost exclusively present in benign lymphocytic choriomeningitis even though the cell count is high.

Tumor cells may be found in occasional cases of medulloblastoma.

I Protein—A globulin test should be done on all fluids examined unless much blood is present. Normal cerebrospinal fluid of either adults or children contains 15 to 45 mg of total protein per 100 cc but gives a negative reaction to the globulin tests recommended. The quantitative test for total protein is sufficiently valuable to justify its determination on all spinal fluids examined.

1 Interpretation—(a) *A one plus globulin reaction* (total protein of 50 to 100 mg). This usually occurs in the conditions listed under G above as producing a white cell count from 10 to 100 or 100 to 500 per c mm and also (without an increase in cell count) in some cases of cerebral arteriosclerosis and brain tumor. It may result from the presence of blood in the spinal fluid.

(b) *A two or three plus globulin reaction* (100 to 500 mg of total protein per 100 cc). This is usually found in the acute types of meningitis, and may occur in paresis, or in syphilitic or tuberculous meningitis.

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(b) *A two or three plus globulin reaction* (100 to 500 mg of total protein per 100 cc). This is usually found in the acute types of meningitis, and may occur in paresis, or in syphilitic or tuberculous meningitis.

(c) *A four plus globulin reaction* (total protein over 500 mg) This is the essential part of the syndrome of Froin. Usually in addition, a deep yellow color is present and massive coagulation occurs. An increase in lymphocytes may or may not be present. This syndrome results from complete spinal subarachnoid block, which in turn is most often due to spinal cord tumor, caries of the spine with tuberculous pachymeningitis externa, or to spinal or cerebrospinal meningitis, particularly of the syphilitic and epidemic forms. It may occur above as well as below a block. The presence of Froin's syndrome is, therefore, usually associated with an absence of increase in pressure on jugular compression in the Queckenstedt test and an Ayala quotient of less than 5. One should always consider the above listed diagnoses if this syndrome is present in part or in whole, but the absence of this syndrome is not a point against any of these diagnoses. Froin's syndrome may occur in some cases of acute polyneuritis or radiculitis of the cauda equina.

J Spontaneous Coagulation—This is due to the presence of fibrinogen. It does not occur in normal fluids but may occur in any of the conditions causing more than a one plus globulin reaction. A very fine pellicle of coagulum is common in tuberculous meningitis, a somewhat heavier coagulum may occur in the purulent types of meningitis, and a massive coagulation occurs in most cases of complete spinal subarachnoid block as a part of Froin's syndrome.

K The Lange Colloidal Gold Test—This is indicated in all cases in which meningo vascular or parenchymatous syphilis of the nervous system, meningitis, or multiple sclerosis are included in the differential diagnosis, therefore, in nearly all neurologic cases.

The typical normal curve shows no change and is read 0000000000,¹ but a change of only 1 in any dilution is not a sufficient basis for conclusions.

1 Interpretation of Abnormal Curves—These are of three types:

(a) *The paretic (Zone I) curve*. This is characterized by the greatest change in the lowest dilutions (first tubes) which usually show complete or almost complete precipitation. A typical curve would read 5554320000, but any curve in which two of the first three dilutions read 4 or 5 is called a paretic curve. This occurs not only in the majority of cases of general paresis but also in some cases of tabes dorsalis, syphilitic meningitis, multiple sclerosis, brain tumor, and in rare instances, in lethargic encephalitis. A paretic curve with a negative spinal fluid Wassermann should cause one to consider multiple

¹ The meaning of the figures used to express results is explained on page 321.

sclerosis or brain tumor, as the spinal fluid Wassermann is almost never negative in paresis, and is positive in 60 to 80 per cent of cases of tabes dorsalis, and 70 to 90 per cent of cases of tertiary neurosyphilis.

(b) *The tabetic (Zone II) curve* In this type the chief changes occur in the third and fourth tubes, less commonly in the fifth tube. A tabetic curve may, therefore, be defined as any curve in which the highest point is in the third, fourth, or fifth tubes, and at least two of these tubes show a reading of 2 or over. Curves which do not go above 2 are not always clinically significant. The tabetic curve rarely shows more complete precipitation than is indicated by 4. This type of curve occurs most constantly and is highest in syphilitic meningitis, it is also present in about 50 per cent of patients with tabes, in most other types of neurosyphilis with the exception of paresis, and in a considerable percentage of cases of multiple sclerosis, brain tumor, encephalitis lethargica, and poliomyelitis in the active stages.

(c) *The meningitic (Zone III) curve* This is characterized by having its peak in the higher dilutions (one of the sixth to eighth tubes). It may be found in any type of meningitis (including the tuberculous) but is less common in syphilitic meningitis. It occurs also in some cases of brain or cord tumor. The peak tends to be less high and to occur in lower dilutions (tube 6) in the tuberculous type than in the acute types.

The various other colloidal tests are discussed in some of the references at the end of the chapter. It is doubtful whether any substitutes yet proposed will displace the colloidal gold solution.

L. Serologic Tests for Syphilis—Complement fixation and agglutination tests for syphilis should be done on all spinal fluids examined and a spinal puncture especially for this purpose is indicated in early syphilis after about six months treatment and again at twenty-four months after completing the continuous treatment. These tests should be done in all patients with latent or tertiary syphilis when they are first seen. The tests should be repeated at intervals on all patients with syphilis. A positive, that is a three to four plus serologic report from a laboratory supervised by a skilled serologist, means potential or actual syphilitic disease of the central nervous system with the exception of the rare false positives that may occur in craniopharyngeoma, or if the cerebrospinal fluid is contaminated with much blood in a patient with a positive serologic test for syphilis in the blood. One to two plus reports call for repetition of the test unless the patient is known to have syphilis. In an untreated patient general paresis may be excluded by a negative test, but ten to twenty per cent of

patients with active tabes dorsalis may have a negative spinal fluid and in treated or old inactive tabes dorsalis forty to sixty per cent may be negative. In syphilitic meningitis, from ten to fifteen per cent of serologic reactions are negative but in vascular neurosyphilis the serologic test for syphilis is negative in forty per cent of the cases.

A positive serologic test for syphilis does not exclude nonsyphilitic disease as the cause of the symptoms which lead the patient to consult the physician.

M Quantitative Chemical Tests—These are indicated in puzzling cases of coma and in suspected meningitis.

1 **Dextrose**—The normal level is 45 to 85 mg per 100 cc if the blood sugar level is normal, or between 45 and 70 per cent of the level in the blood. The values are the same in children. It is decreased (to 0 or a few mg so that 15 drops fail to reduce 5 cc of Benedict's solution) in the later stages of the acute purulent forms of meningitis and to below 40 mg in many cases of tuberculous meningitis. It is usually normal in neurosyphilis. It is increased in diabetes mellitus and other types of hyperglycemia in proportion to the elevation of the blood sugar but still remains at a lower level. This increase may be of diagnostic value in cases of coma if other causes of hyperglycemia (particularly uremia and cerebral hemorrhage) are excluded. In lethargic encephalitis it is reported to be somewhat increased, the fundamental change appearing to be increased permeability of the choroid plexus permitting the dextrose level to approach more closely to that of the blood. This is best detected by estimations of dextrose in blood and cerebrospinal fluid taken simultaneously. If the cerebrospinal fluid sugar level is more than 80 per cent of the blood sugar level such an increased permeability is suggested. It must be remembered, however, that as dialysis is a relatively slow process there is a definite lag in the change of concentration of substances in the spinal fluid as compared to changes in their concentration in the blood stream. Hence it is possible for the dextrose level (or level of any other diffusible substance) to be higher than that of the same substance in the blood stream if a recent rapid drop in the concentration of that substance has occurred in the blood stream and, vice versa, a much lower percentage of such a substance will be present in the spinal fluid than one would expect from its level in the blood immediately after a rapid rise of the concentration in the blood.

In cases of coma a marked increase in spinal fluid sugar (diabetes mellitus, uremia, cerebral trauma) may be very rapidly detected by the technic given on page 521.

2 **The Chloride Estimation**—This is of some value in differentiating a generalized from a localized meningitis and in differentiating the tuberculous from the syphilitic forms. The results are expressed as mg of sodium chloride per 100 cc. In normal adults the level is extremely constant at 720 to 750 mg. In normal children much wider variations occur and results must be below 625 mg or over 760 mg to be regarded as abnormal. Low values from normal down to 625 mg in adults or to 575 mg in children occur in all types of acute generalized meningitis except the syphilitic. Still more marked decreases may occur in tuberculous meningitis down to 550 mg in adults and to 500 mg in children. The lowering of the chloride does not occur in abscess, tumor, hydrocephalus or encephalitis.

Both the chlorides and dextrose may be normal in early cases of all types of meningitis, so that only deviations from the normal should be given much weight

3 **The Urea Nitrogen**—This follows the level in the blood almost exactly and may be determined by the same method. It has no advantages over the estimation in blood except that it saves a venipuncture if the cerebrospinal fluid is being taken anyway, as in coma. An estimation of 60 mg or over per 100 cc would be compatible with a diagnosis of uremic coma. A significantly lower estimation would exclude it.

4 **The Mercury Combining Power of the Spinal Fluid**—This will give a much simpler but somewhat less accurate clue to the diagnosis of uremic coma. If 2 per cent mercuric chloride is used to titrate 1 cc of spinal fluid as directed a titration of 3 cc or over indicates significant nitrogen retention and of 4 cc or over a degree of nitrogen retention compatible with the diagnosis of true uremia.

5 **The Alkali Reserve of the Spinal Fluid**—This may be determined directly on the cerebrospinal fluid in cases of coma. The normal values, technic, and interpretation are the same as for plasma.

6 **"Creatinine" in the Cerebrospinal Fluid**—Normal values are 0.5 to 2.0 mg per 100 cc. It appears to follow the level in the blood very closely and as the technic on cerebrospinal fluid is extremely rapid and simple it should prove of value in the rapid diagnosis of uremic coma. It may be estimated by the same technic as for blood either by using the colorimeter or by direct comparison with the permanent dichromate standards. An estimation of 3 mg or over would be compatible with uremic coma, and of 5 mg or over per 100 cc would make this diagnosis almost certain.

7 **Other Constituents of the Spinal Fluid**—Normal calcium of the cerebrospinal fluid is 4 to 6 mg per 100 cc or 40 to 60 per cent of the total serum calcium. It corresponds to the diffusible calcium of the serum and is decreased or increased in the conditions listed as causing alterations in total calcium except those in which the alteration in calcium is associated with an alteration in the level of the plasma proteins.

Spinal fluid phosphate expressed as phosphorus is 1.25 to 2.00 mg per 100 cc. Cholesterol is not found in spinal fluid. If bromide poisoning is suspected a determination may be made on the spinal fluid. The value multiplied by 3 will correspond roughly to the blood bromide level.

N Special Tests for Tuberculous Meningitis—The three tests proposed for the diagnosis of tuberculous meningitis have not yet been sufficiently studied to justify a statement as to their reliability.

II COMA²

This demands prompt treatment, hence, the cause should be determined as soon as possible. It is important to have a systematic

¹ Gleich M, and Hartstein, A. The Levinson Test in Tuberculous Meningitis. *Am J Dis Child* 43: 1077-1085 (May) 1932.

Food A G and Forsyth Anna. The Laboratory Diagnosis of Tuberculous Meningitis. *Am J Clin Path* 3: 45-54 (Jan) 1933.

Greenthal R M. Nitrite Reaction as Diagnostic Test in Influenzal Meningitis. *Am J Dis Child* 40: 569 (Sept) 1930.

Lichtenberg H H. The Tryptophan Test in Tuberculous Meningitis. *Am J Dis Child* 43: 32-39 (Jan) 1932.

² Solomon P, and Aring C D. The Differential Diagnosis in Patients Entering the Hospital in Coma. *J A M A* 105: 7-12 (July 6) 1935.

plan of examination of these patients well in mind so that no time will be lost. Such a plan for the laboratory phase of the examination is given below. The common causes of coma are head injuries, cerebral vascular accidents including hemorrhage, thrombosis, or embolism, uremia, diabetic coma, poisoning especially with alcohol, morphine, carbon monoxide, barbiturates, lead, and many others, hypoglycemia, cerebral edema, and tumors or infections of the brain or meninges. There are many other causes but this is a sufficiently complete list for the purposes of this text.

The tests on the urine, cerebrospinal fluid and blood interpreted below should be done as soon as possible. For the greatest efficiency all three fluids should be secured and examined by assistants while the preliminary physical examination is completed and therapy is started. In some instances the physical examination or a portion of this laboratory examination will indicate so clearly the cause, that further laboratory studies are not indicated. Such cases are unusual. The common mistake is to stop before a sufficiently complete study has been done. Two conditions not infrequently co exist. A man may have diabetes and develop a cerebral hemorrhage, he may develop uremia while he is drunk, or head injuries may be the result of a fall due to coma, rather than the primary cause. Hence, a complete and thorough examination is desirable even though an apparent cause has been found.

A Urine Examination—This is indicated in all cases. The patient should be catheterized as soon as possible. The urine examination alone is never sufficient to establish the diagnosis, as albumin and casts may be due to various poisons, passive congestion, or accidental co existence of renal disease, while glycosuria is common after cerebral trauma or may be due to a condition unrelated to the cause of the coma in the particular patient. Ketosis is frequently due to insufficient carbohydrate intake. Uremic or diabetic coma is unlikely if the urine is normal. If the patient is known to have diabetes mellitus and dextrose is found in the urine, dextrose with insulin may be started as soon as blood has been drawn for chemical study, since it will do no harm even if it be found later that the coma is not due to diabetic acidosis. In such a patient, if dextrose is absent from the urine, blood for a sugar estimation should be drawn and sterile dextrose solution should be given intravenously at once as the coma may be due to hypoglycemia.

B The Cerebrospinal Fluid Examination (see the first of this chapter for a more detailed discussion) —The important points to note in cases of coma are

1 **The Pressure**—Increase is the rule in head injuries, uremia, diabetic coma, alcoholism, meningitis, cerebral hemorrhage, etc., and therefore, is of little differential value

2 **The Appearance**—A uniformly bloody fluid is usually due to hemorrhage communicating with the subarachnoid space, either directly or indirectly through the ventricles or tears in the brain substance or meninges. Such hemorrhages are most often due to arteriosclerosis, hypertension, congenital or syphilitic aneurysm of the cerebral arteries, or to trauma. The presence of blood is an indication for stereoröntgenograms of the skull for fracture. Its absence does not exclude intracranial hemorrhage or skull fracture.

(a) **Cloudy Fluid**—This is usually due to acute purulent meningitis, but any of the causes of a cell count over 500 per c. mm. may give it. Owing to the frequency of the meningococcus type and the importance of starting treatment early, it is usually advisable to give antimeningococcus serum intraspinally while the needle is still in place and to start parenteral administration of sulfanilamide. If further study shows it to be of another type no harm has been done, and if it proves to be of the meningococcus type precious time has been saved. As soon as the organism has been identified, treatment appropriate to the infecting organism may be instituted.

3 **The Cell Count, Differential Count and Examination of Stained Smears for Organisms**—This should be done at once if the fluid is cloudy or the history or physical findings suggest meningitis or syphilis of the nervous system. In other cases it is best deferred (but not longer than an hour) until uremia and diabetic coma have been ruled out.

4 **Reduction of Benedict's Solution**—This should be tested at once on all nonbloody fluids. A four plus reduction with fifteen drops of spinal fluid indicates diabetes mellitus, a two or three plus reduction may be due to any cause of hyperglycemia. Any increase in reduction is an indication for an immediate alkali reserve estimation and a quantitative sugar estimation on the blood or cerebrospinal fluid. Absence of reduction is usual in the purulent and tuberculous forms of meningitis.

5 **The Mercury Combining Power, "Creatinine," or Urea Nitrogen**—One or more of these estimations should be done at once on all clear fluids from patients whose urine showed albumin, casts, or blood or if no urine was obtainable, and on all fluids from patients who are suspected for any reason of having uremia. Increase in any of these substances is an indication to start therapy for uremia, while

the alkali reserve, urea nitrogen and "creatinine" are being determined in the blood

When the above immediate indications have been met and emergency therapy has been instituted the cerebrospinal fluid should be further studied according to the indications outlined under I at the first of this chapter

C Blood Examination—The alkali reserve, blood urea nitrogen, and blood sugar should rarely be omitted on patients in coma even though an apparent cause for the coma has been found. Blood for these examinations should be drawn before treatment is instituted. Tests for alcohol, carbon monoxide, bromides, or other poisons are often indicated

1 The Alkali Reserve—This is the most important test on a patient in coma. An alkali reserve above 30 excludes diabetic coma. A low alkali reserve may occur not only in uremic coma and in patients with impaired renal function without uremia but from anhydremia in diabetic coma, and in patients who have been comatose for some time from any other cause. Alkalosis is also an important cause of coma in patients with impaired renal function or in patients who are losing hydrochloric acid or receiving alkalies, and is a common result of overtreatment of acidosis if this treatment is not controlled by frequent alkali reserve determinations

2 The Blood Urea Nitrogen—A blood urea nitrogen of over 60 mg per 100 cc makes true uremia the most probable cause of the coma. A lesser increase is common in patients with cerebral edema from acute glomerular nephritis, and may occur in any patient who has been in coma for some period of time, due to anhydremia from deficient fluid intake

3 The Blood Sugar—A blood sugar above 200 mg per 100 cc is the rule in diabetic coma and may be as high as 1,600 mg. Moderate elevation of the blood sugar to 150 to 250 mg per 100 cc is common in patients with head injuries or cerebral vascular accidents. If the blood sugar level is below 50 mg per 100 cc, hypoglycemia is the most probable cause of the coma

4 The Icterus Index—An increase in the icterus index in the first few days after a cerebral vascular accident favors hemorrhage rather than encephalomalacia

5 Test for Alcohol in the Blood¹—The presence of alcohol on the breath does not prove that coma is due to intoxication, but a con

¹ Bogen E. Drunkenness. Quantitative Study of Acute Alcoholic Intoxication. *Am J M Sc* 176: 153-167 (Aug) 1928

Bogen E. Tolerance to Alcohol. Its Mechanism and Significance. *California & West Med* 44: 262-271 (Apr) 1936

centration in the blood of over 200 mg of alcohol per 100 cc indicates definite intoxication and over 300 mg is compatible with coma on the basis of alcoholic intoxication

6 Tests for Carbon Monoxide Hemoglobin in the Blood — This should be determined at once when there is a history suggesting exposure to carbon monoxide or if the skin and mucous membranes have a distinctive color, usually described as "cherry" red. A concentration up to 10 per cent may occur in city dwellers, after cigarette smoking, or in anyone who has driven in traffic. This does not produce symptoms. A concentration up to 20 per cent may occur in taxi drivers, traffic policemen, or others exposed to heavy traffic conditions, but rarely causes symptoms. Concentrations of 20 to 30 per cent may produce headache, nausea and dizziness. Concentrations of 30 to 40 per cent always produce these symptoms. Concentrations above 40 per cent may lead to coma and above 50 per cent always lead to coma. It is rare for a patient to recover if more than 80 per cent of the hemoglobin is in the form of carbon monoxide hemoglobin. Since, if the patient lives and is removed from the atmosphere of carbon monoxide, the concentration will be below 10 per cent within 9 hours after a maximum exposure and within 2 to 3 hours after a minimal exposure, the determination to be of value must be done as soon as possible after the patient is removed from this environment. From one third to one half of the carbon monoxide present at the beginning of any hour disappears by the end of that hour. Consequently, the original concentration can be roughly figured from the concentration at the time of the determination if the time since the patient was removed from exposure is known. After death, carbon monoxide hemoglobin breaks down very slowly, however, and determinations for medicolegal purposes or to determine the cause of death may be of value for long periods of time after death if exposure of the body to carbon monoxide after death has been excluded. The method described on page 415 is much simpler than the usually recommended methods for this determination.

7 Serum Bromide Determination — Since seven per cent of admissions to hospitals for the insane are cases of bromide intoxication, this test is indicated routinely in all psychiatric cases. Since bromide intoxication may simulate or complicate various neurologic disorders, this test is indicated when neurologic symptoms occur. A bromide estimation is also indicated in cases of coma of obscure etiology. Bromide therapy should be controlled by frequent bromide estimations, and this test should be done when a history of prolonged use of the drug is obtained. Many proprietary preparations contain bromides and the

name of the preparation does not always suggest the presence of bromides

(a) *Interpretation* —A trace (10 to 15 mg per 100 cc) of bromide is normally present in the serum. When the serum bromide is above 100 mg, toxic symptoms are likely to occur. When the level is above 175 mg, psychotic symptoms are usually present. If the level is above 275 mg, a fatal outcome is probable. Treatment of bromide intoxication by high sodium chloride intake should be controlled by frequent bromide estimations.

III SUMMARY OF THE DIFFERENTIAL DIAGNOSIS OF THE DISORDERS OF THE CENTRAL NERVOUS SYSTEM AND OF THE CAUSES OF COMA

There is no logical order in which to discuss these conditions so they are given approximately in their order of frequency as causes of coma. At times, syndromes rather than specific diseases are given to conserve space.

A *Cerebral Anemia* —This is the most common cause of coma and rarely offers difficulty in diagnosis. It includes ordinary fainting, shock, large acute hemorrhage from any cause, profound anemia, the coma of moribund patients, and the coma of Stokes Adams disease, aortic stenosis and paroxysmal tachycardia. The diagnosis is usually based on the history and physical findings of low blood pressure, sweating and rapid pulse. The cerebrospinal fluid is normal except for decreased pressure. The laboratory findings in shock and acute hemorrhage are given in other chapters. Cerebral anemia is the only form of coma in which it is desirable to have the head low. When this has been excluded the head may be elevated.

B *Epilepsy* —Postepileptic stupor is a common cause of coma. Laboratory findings in the blood, cerebrospinal fluid and urine are usually normal. The condition is recognized by the history, evidence of previous injuries, and by exclusion.

C *Hysteria* —The laboratory and physical findings are normal. Incontinence of urine does not occur. Injuries from convulsions or falling are absent. Other causes of coma should be excluded.

D *Alcoholism* —An alcohol content in the blood or urine above 300 mg per 100 cc establishes the diagnosis of alcoholic intoxication but to prove it is the sole cause of coma, other conditions must be excluded. The presence of an alcoholic odor on the breath does not exclude other causes of coma.

E *Head Injuries* —Blood is often present in the cerebrospinal fluid with or without skull fracture. The cerebrospinal fluid pressure

is usually elevated. It is most important to exclude the possibility of the injury being due to coma from one of the other causes listed.

F Cerebral Vascular Accidents—Hemorrhage, thrombosis or embolism are common causes of coma. The presence of blood in the cerebrospinal fluid indicates cerebral hemorrhage usually with a grave prognosis, but its absence does not exclude this as the explanation for a hemiplegia. Pleocytosis may occur and is more common in hemorrhage than in encephalomalacia. The pressure is more often increased in hemorrhage than in encephalomalacia. An increase in the icterus index, which begins four to eight hours and persists for a week or two after the accident, occurs in hemorrhage but not in encephalomalacia. In many instances accurate differentiation is impossible. Transient hemiplegia of from a few minutes to a few hours duration may be due to focal edema.

G Poisoning—Carbon monoxide, morphine, barbiturate, bromide, and phenol are common causes of coma. Mercury poisoning leads to uremia, acidosis or alkalosis. The stomach should be washed out and the contents examined toxicologically. Tests for carbon monoxide hemoglobin, serum bromide, or barbiturates in the urine should be performed if indicated by the history. In carbon monoxide or phenol poisoning the cerebrospinal fluid pressure is usually increased.

H Cerebral Edema—This may occur in eclampsia, in all types of kidney disease associated with hypertension, after head injuries and in many types of poisoning. The cerebrospinal fluid pressure is increased without marked alteration in the proteins or cell count unless bleeding is associated. It also occurs in the most severe cases of congestive heart failure. In the cases associated with hypertension, retention of urea nitrogen and acidosis may also be present. The therapy is hypertonic dextrose, sucrose or sorbital administered intravenously.

I Acidosis—The causes of acidosis have been listed previously. *Coma is not due to acidosis unless the alkali reserve figure is less than 30.* The two most common forms of coma due to acidosis are those of diabetes and of impaired renal function.

1 Diabetic Coma¹—The alkali reserve is always low and the blood sugar is usually, though not always, elevated, and the urea and

¹ Joslin E P, Root H F, White Priscilla, Marble A and Joslin, A P. Diabetic Coma. Arch Int Med 59: 175-195 (Feb.) 1937.

Baker T W. A Clinical Survey of One Hundred and Eight Consecutive Cases of Diabetic Coma. Arch Int Med 58: 373-406 (Sept.) 1936.

Dillon E S and Dyer W W. Factors Influencing the Prognosis in Diabetic Coma. Ann Int Med 11: 602-617 (Oct.) 1937.

"creatinine" are normal. Marked ketosis and glycosuria are usually present. *If the alkali reserve is not below 30, diabetic coma is not present.*

2 **Acidosis of Impaired Renal Function**—This results from an excess of acid over basic radicles in the diet or therapy. It is frequently mistaken for true uremia which it may complicate. Hypertension is usually present and the blood urea nitrogen is always over 20 mg per 100 cc. Ketosis is usually absent. The urine shows the changes characteristic of the condition causing the impaired renal function.

J **Uremia**—True uremia is not present if the blood urea nitrogen is not over 60 mg per 100 cc. The causes have been given. It is important to differentiate uremic coma from cerebral edema and from acidosis of impaired renal function. Do not conclude that the patient who has anhydremia from having been in coma for a day or two without adequate fluid intake and who has, therefore, some elevation of the blood urea nitrogen has true uremia.

K **Alkalosis**—The causes have been listed. Coma from alkalosis is unlikely to occur unless the alkali reserve is above 100. The symptoms simulate those of acidosis. The most common cause is excessive ingestion of alkalis or loss of hydrochloric acid from the stomach especially if either is complicated by impaired renal function. The treatment is slow intravenous administration of the calculated amount of sterile 1 per cent hydrochloric acid and removal of the cause. The syndrome of anhydremia is often associated.

L **Hypoglycemia**—The causes of hypoglycemia have been given. The blood sugar is usually under 50 mg per 100 cc if the patient is in coma. Since it may occur in patients with diabetes it is important to exclude this before giving insulin without dextrose. Sugar may be present in the urine in patients with diabetes who have accumulated considerable urine in the bladder before the blood sugar fell but in the majority of patients with hypoglycemic coma sugar is absent. Intravenous dextrose solution produces prompt recovery.

M **Meningitis**—Stiffness of the neck is the most dependable clinical sign, but may be present in subarachnoid hemorrhage, poliomyelitis and arthritis of the cervical spine. The cerebrospinal fluid findings are most important. In all types of meningitis the cerebrospinal fluid pressure and Ayala's quotient are increased unless subarachnoid block has occurred in which case the pressure and Ayala's quotient are low.

1 **Acute Purulent Meningitis**—The pressure is increased, the fluid is cloudy, the cell count is usually over 500 with a predominance of neutrophils, the Lange curve is usually in zone II or III, and the

causative organism, the meningococcus, pneumococcus, influenza bacillus, streptococcus or staphylococcus, may be found in direct smears or cultures. The protein is usually between 100 and 1,000 mg per 100 cc, and dextrose, except within a few hours of the onset, is usually under 20 mg per 100 cc of spinal fluid.

2 **Aseptic Meningitis**—This gives the same picture as acute purulent meningitis except that organisms are absent, the cell count is seldom over 2,000 per c mm, and the protein content is seldom over 500 mg per 100 cc. The dextrose and chlorides are normal. The presence of this syndrome indicates an immediate search for infection in the middle ear, mastoid, or cranial bones with prompt steps to secure adequate drainage and specific therapy with sulfanilamide or related compounds. The syndrome may also result when hemorrhage, abscess or tumors approach sufficiently near the surface of the meninges or ventricles.

3 **Tuberculous Meningitis**—This is most common in infants but may occur at any age. It is often associated with generalized miliary tuberculosis. The fluid is usually clear or slightly opalescent, the cell count is usually between 100 and 700 per c mm with 40 to 90 per cent of the cells lymphocytes, ordinary cultures and smears reveal no organisms, a fine coagulum which contracts to form a pellicle on standing occurs, the dextrose is usually under 40 mg per 100 cc except within 24 hours of the onset, and the sodium chloride level of the cerebrospinal fluid is more often under 600 mg per 100 cc than in other forms of meningitis. The higher lymphocyte count and the absence of organisms differentiate it from acute purulent meningitis and the lower dextrose, chlorides, and negative serologic test differentiate it from syphilitic meningitis. The diagnosis is confirmed by finding the tubercle bacillus in Ziehl-Neelsen stains or by culture and guinea pig inoculation, but the patient is usually dead before these results are available. The Lange curve is usually in zone II or III.

4 **Syphilitic Meningitis**—The cell count is usually between 100 and 1,000 per c mm with lymphocytes usually above 60 per cent. The protein is usually between 50 and 100 mg per 100 cc, and the Lange curve is usually in zone I or II.

5 **Lymphocytic Choriomeningitis**¹—This virus disease is characterized by a cell count of 50 to 1,000 per c mm, nearly all lymphocytes, with normal dextrose and little or no elevation of the protein. The Lange curve is usually normal. Organisms are not found in the usual smears and cultures.

¹ Viets, H. R. and Warren, S. Acute Lymphocytic Meningitis. J. A. M. A. 108: 357-361 (Jan. 30) 1937.

N Subarachnoid Hemorrhage—This should be considered when a young person without apparent cause suddenly develops severe headache or coma with stiff neck. Blood is always present in the fluid in the early stages and the pressure is usually increased. The protein content and pleocytosis are proportional to the amount of blood present in the early stages and are increased out of proportion to the number of red cells present after the first few hours. It usually results from rupture of a congenital aneurysm of one of the arteries constituting the circle of Willis.

O Subdural Hematoma—This should be considered when a second loss of consciousness develops a few days to a few weeks after apparent or partial recovery from a head injury. The cerebrospinal fluid pressure is usually increased, the fluid is often yellow or bloody, and the protein is usually between 50 and 500 mg per 100 cc. The fluid may be normal in all respects and the diagnosis has to be made by the history and physical examination.

P Intracranial Tumors—The signs to be described apply to all types of tumors, benign or malignant, primary, metastatic or encroaching from adjacent areas, and to other space-occupying lesions such as internal hydrocephalus, abscess or gumma. There is a tendency to increased cerebrospinal fluid pressure with decreased Ayala's quotient if the tumor is of large size or is producing internal hydrocephalus.

1 Supratentorial Tumors—The cell count is usually normal and rarely over 100 per c mm, lymphocytes predominate. The protein is usually normal unless the ventricle or subarachnoid space is invaded. The sugar and chloride contents are normal as is the Lange curve in those patients with normal protein. The Lange curve may show second or third zone reactions in many cases and, rarely, a first zone reaction.

2 Infratentorial Tumors—*Lumbar or cisternal punctures are contraindicated.* Ventricular puncture is safer. If, because of failure to make a clinical diagnosis or because little or no papilledema exists, the cerebrospinal fluid is examined, the pressure is usually increased. The protein is usually over 100 mg per 100 cc in acoustic neuromas or in other tumors secondary to neurofibromatosis and is usually normal in cerebellar tumors.

Q Cord Tumors—Under this heading are included not only true neoplasms originating in the spinal cord, but all other neoplasms encroaching on the vertebral canal and space-occupying lesions such as herniation of the intervertebral discs, hypertrophy of the ligamentum flavum, pachymeningitis externa and abscesses or metastatic tumors of the vertebral bodies. All these may produce the characteristic

symptoms of *spinal subarachnoid block* This is characterized by a normal or low pressure with no elevation in the Queckenstadt test if the block is complete and delayed response and especially delayed or incomplete return to normal in the blood pressure cuff modification of the Queckenstadt test These signs do not occur if the site of the puncture is above the block Troin's syndrome will be present if fluid is obtained either below or just above a complete block The fluid is usually normal if only slight block exists In those cases with an increased protein content, any type of Lange curve may occur

R Encephalitis—In all types of encephalitis the pressure is usually normal, the cell count is not over 100 per c mm and often is normal, the cells are all lymphocytes, the protein is slightly if at all increased, and the sugar and chlorides are usually normal These are virus diseases so no organisms are found by ordinary methods Normal sugar content and negative serologic tests for syphilis differentiate encephalitis from tuberculous and syphilitic meningitis The Lange curve is usually normal but zone II curves are seen occasionally and in rare instances a zone I curve occurs

S General Paresis—This important disease of the brain occasionally results in the sudden onset of coma from acute cerebral edema in which case the pressure is usually increased In most cases of general paresis the pressure is normal, the cell count is 20 to 150 per c mm, mostly lymphocytes, the protein is between 50 and 100 mg per 100 cc, the sugar and chlorides are normal, and almost 100 per cent of untreated patients show a first zone Lange curve and 4 plus serologic tests for syphilis in the cerebrospinal fluid

T Tabes Dorsalis—This important disease does not directly produce coma but one of the commonest causes of death from tabes dorsalis is uremia from pyelonephritis secondary to paralysis of the bladder The cerebrospinal fluid pressure is normal, the cell count is usually between 10 and 60 per c mm, all the cells are lymphocytes, the protein is normal or slightly increased, the sugar and chlorides are normal, and the Lange curve is typically zone II but some patients with tabes have a first zone reaction, and quiescent or treated patients often show no alteration in the Lange Serologic tests for syphilis are positive in from 60 to 80 per cent of active cases

U Multiple Sclerosis—This does not lead to coma directly but, as with tabes dorsalis, pyelonephritis with uremia is a common termination The cerebrospinal fluid is usually entirely normal but a cell count up to 40 per c mm, all lymphocytes, and a slight increase in protein is not uncommon A zone I Lange curve occurs in about

one fourth of the cases and a zone II Lange in another one fourth. A first zone Lange with negative serologic tests for syphilis should lead one to consider the diagnosis of multiple sclerosis.

V Anterior Poliomyelitis—This usually gives a stiff neck as does meningitis and in the preparalytic stage may be difficult to differentiate from tuberculous meningitis. In the preparalytic stage clinical diagnosis may be impossible without the cerebrospinal fluid examination. The diagnosis must be made on the basis of the spinal fluid changes in a patient with acute illness in the presence of an epidemic. The pressure is normal, the appearance clear or slightly opalescent, the cell count is usually between 20 and 500 per c mm but in rare instances may be over 1,000 with lymphocytes as a rule predominating. The protein is usually between 50 and 200 mg per 100 cc but may be normal. After repeated punctures protein levels above 200 mg sometimes are found. Sugar and chlorides remain normal which aid in differentiating it from acute tuberculous meningitis. The Lange curve is usually normal but zone II curves may occur. Coma occurs from respiratory paralysis with asphyxia or from an ascending poliomyelitic encephalitis or from intercurrent disease, but poliomyelitis rarely has to be considered in the differential diagnosis of acute coma.

W Polyneuritis—The occasionally increased cell count and frequently increased protein in the cerebrospinal fluid make it necessary to include this condition. It is not a cause of coma but may be due to lead or arsenical poisoning either of which may produce encephalopathy or uremia, or to pellagra in alcoholics, and it may at times simulate clinically tabes dorsalis. The Lange curve in polyneuritis may show any type of curve but is usually normal.

ADDITIONAL REFERENCES

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- Flexner L B. *The Chemistry and Nature of the Cerebrospinal Fluid*. *Physiol Rev* 14: 161-187 (April) 1934.
- Gilligan D R, Volk Marie C and Blumgart H L. *Observations on the Chemical and Physical Relation between Blood Serum and Body Fluids. II. The Chemical Relation between Serum and Edema Fluids as Compared with that between Serum and Cerebrospinal Fluid*. *New England J Med* 210: 896-903 (April) 1934.
- Clauser J. *The Cerebrospinal Fluid of Premature Infants With Special Reference to Intracranial Hemorrhage and Pigmentation*. *Child*, 36: 195-247 (Aug) 1928.

CHAPTER XI

PREGNANCY AND ITS COMPLICATIONS¹

It is important to bear in mind that almost any condition to which nonpregnant women of child bearing age are subject may also affect pregnant women. Of such conditions pregnant women are particularly susceptible to pyelitis, hydronephrosis, nephritis, nephrosis, hyperthyroidism, acute yellow atrophy of the liver, and osteomalacia. They are apt to develop exacerbations of a pre-existing chronic diffuse nephritis, hypertensive cardiovascular renal disease or pulmonary tuberculosis. As these are more fully discussed in other chapters, only conditions peculiar to pregnancy will be discussed in detail here.

I. RESUMÉ OF THE ESSENTIAL POINTS IN THE PHYSIOLOGIC AND BIOCHEMICAL CHANGES OCCURRING IN NORMAL PREGNANCY²

In addition to the extra strain on the maternal assimilative and eliminative functions incident to supplying the materials for the development of the fetus, placenta and membranes and to the excretion of the waste products of fetal metabolism, profound alterations occur in the metabolism of the mother herself. Almost immediately after impregnation a hormone derived from the chorionic tissue of the placenta similar in action to the gonadotropic hormone of the anterior lobe of the pituitary may be demonstrated in the patient's urine. This excretion of anterior pituitary-like hormone persists throughout pregnancy and is soon associated with increased formation and excretion in the urine of estrogenic substance. Increase in size of the thyroid, and formation of the corpus luteum of pregnancy suggest that other less fully studied endocrine changes also occur.

In the first three months of pregnancy the renal threshold for dextrose is lowered, resulting in an increased tendency to alimentary glycosuria. Ketosis is frequently present but if it is controlled by increased carbohydrate intake, it is the author's impression that the so-called "physiological" nausea of pregnancy is less likely to occur and the pernicious vomiting of pregnancy is decreased in frequency.

In the latter months of pregnancy the urine volume increases about one fourth, the blood cholesterol values are high, the serum calcium is low, and the fibrinogen content of the plasma increases with a corresponding increase in the

¹ Stander H. J. The Toxemias of Pregnancy. *Medicine* 8: 1-157 (Feb) 1929. This monograph touches on practically every subject discussed in this chapter.

Ehrenfest H. The Normal and Pathologic Physiology of Pregnancy. *Am J Obst and Gynec* 12: 58-76 (July) 1926.

² See the additional references at the end of the chapter.

sedimentation rate of the red cells The basal metabolic rate¹ begins to increase in the thirty-fourth week, attaining a maximum average of plus 15 per cent with a few results as high as plus 25 to plus 35 per cent just before delivery It reaches normal again about 10 days after delivery The rise seems to be due chiefly to the extra metabolism of the fetus

The blood sugar is usually within the normal limits but the average is slightly lower than normal A hypoglycemia may be associated with even the so called "physiologic" morning sickness of early pregnancy and constitutes a definite therapeutic indication The blood urea nitrogen tends to be in the lower normal range, and estimations as low as 5 mg per 100 cc have been reported² The urea clearance test³ is normal until the last two months of pregnancy Investigators differ as to results in the last two months Some claim that it is decreased and others that it is increased Until the normals have been established more definitely, a urea clearance test cannot be regarded as of definite significance unless it is below 40 per cent of normal Non-protein nitrogen is normal or low Creatinine is normal and uric acid corresponds to high normal figures The carbon dioxide combining power of the plasma tends to be lowered so that the majority of results in the last few weeks of pregnancy fall between the alkali reserve figures of 40 to 60 instead of between 50 and 80 as in non-pregnant women It returns to normal after delivery without treatment The cause of the lowered alkali reserve is disputed, some believing it to be compensatory for a loss of carbon dioxide and others believing it to be due to ketosis The pH of the serum is within normal limits

Creatine is excreted in the urine during the latter part of pregnancy and for a while after delivery

Since creatine is not discussed elsewhere in this book it is worthy of note that it occurs also in urines of young children of both sexes, in normal women during menstruation, and in diseases associated with muscular wasting (starvation, fevers, muscular atrophies and dystrophies Simmond's disease etc) It has also been reported in the urine of insane women, in cases of myositis fibrosa, myotonia atrophica hyperthyroidism cretinism achondroplasia and infantilism Its estimation does not aid diagnosis It does not occur in the urine of normal men nor in the urine of normal women between the menstrual periods Excretion of creatin in the urine is increased by the therapeutic administration of glycine (or gelatin) to patients with muscular dystrophy

Lactose may appear in the urine during the latter months of pregnancy and throughout lactation.

¹ Plass F D and Yoakam W A Basal Metabolism Studies in Normal Pregnant Women with Normal and Pathologic Thyroid Glands *Am J Obst and Gynec* 18 556-568 (Oct) 1929

² Denis W King F I and Briggs F The Ratio of the Urea Nitrogen to Total Nonprotein Nitrogen in the Blood in Normal Pregnancy *Am J Obst and Gynec* 17 386-391 (March) 1929

Cadden J F and Farris A M The Nonprotein Urea and Rest Nitrogen of the Blood During Labor and the Puerperium *Am J Obst & Gynec* 36 77-84 (July) 1938

³ Cantarow A and Ricchiuti G Urea Clearance Test in Pregnancy *Arch Int Med* 52 637-646 (Oct) 1933

Hurwitz D and Ohler W R Urea Clearance Test in Toxemia of Pregnancy *J Clin Invest* 11 2119 (Nov) 1932

The total blood volume increases progressively to a value about 25 per cent above the normal. The increase affects chiefly the plasma, and probably accounts for at least a part of the usual apparent decrease in red cells and hemoglobin. The white cell count¹ increases, averaging about 10,000 and cannot be considered definitely abnormal unless values over 15,000 are secured during the first eight months, or over 35,000 just after delivery. It returns to normal during the puerperium. A relative and absolute neutrophilic leukocytosis with a relative increase in immature forms is associated.

In normal pregnancy the red cell count, cell volume, and hemoglobin content of the blood decrease progressively to a low level, averaging about 15 per cent below the average normal for women, by the sixteenth to twenty-second week. They remain at the same level until the thirty-fourth week, at which time there is a sharp rise followed by a fall lasting until term and affecting chiefly the hemoglobin. A further drop of about 5 per cent occurs on the first day post partum due to the physiological loss of blood, but this is made up within a few days. Following this there is a more gradual rise affecting first the red cells, then the cell volume and last the hemoglobin, bringing all values back to normal by about the sixth month after delivery.

All of the above changes are usually described as the physiological accompaniments of normal pregnancy. In the author's opinion, however, the low blood sugar, low blood calcium, the anemia and the lowered alkali reserve are undesirable. More marked changes of similar type are characteristic of the complications which pregnant women are prone to develop. Therefore, it is probable that active therapy designed to correct these minor deviations may prevent the development of toxemias, anemias, dental caries, or osteomalacia, which might otherwise occur in a certain proportion of these cases.

II THE DIAGNOSIS OF PREGNANCY BY LABORATORY METHODS²

All other tests for the diagnosis of pregnancy have been superseded by the Friedman modification of the Aschheim Zondek test.

A The Friedman Modification of the Aschheim-Zondek Hormone Test³—This depends on the detection of the anterior pituitary like hormone in the urine. Enormous excretion of this hormone begins constantly within 10 days after the expected date of onset of the first missed menstrual period after conception and persists throughout pregnancy and for the first 2 to 4 days of the puerperium. Positive tests have been reported in pregnancy before the expected

¹ Carey J. B., and Litzenberg, J. C. Total Leukocyte Counts in Human Blood During Pregnancy. *Ann Int Med* 10: 25-29 (July) 1936.

² Davis M. and Walker, Elizabeth W. Modern Laboratory Methods for the Early Diagnosis of Pregnancy. *New England J Med* 206: 173-183 (Jan 28) 1932.

³ Mathieu A., Palmer A., and Holman A. The Friedman Pregnancy Test. *Northwest Med* 31: 215 (May) 1932.

Parvey B. Recent Advances in the Physiology of Reproduction in Relation to the Zondek Aschheim Test for Early Detection of Pregnancy. *Endocrinology* 16: 225-241 (May-June) 1932.

Goldberger M. A., Salmon, U. J. and Frank R. T. Value of Friedman Test in Diagnosis of Intra Uterine and Extra Uterine Pregnancy. *J A M A* 103: 1210-1212 (Oct 20) 1934.

date of the first missed menstruation Since retention of living placental tissue is associated with a persistent positive test even after delivery or death of the fetus, this test may be used as a test for incomplete removal of the placenta In hydatidiform mole, chorion epithelioma,¹ and chorion epitheliomatous teratoma (usually of the testicle), a very strongly positive test is the usual finding Quantitative methods have been devised for expressing the units of anterior pituitary like gonadotropic hormone in the urine They involve the use of a large number of animals and are rarely necessary for diagnosis The results may be misleading because chorionepithelioma and the various testicular tumors do not always result³ in the excretion of excessively high quantities of anterior pituitary like gonadotropic hormone, while such excessive excretion does occur in normal early pregnancy⁴ for a week or so, and in most patients with pernicious vomiting Incomplete removal of the tumors or the presence of metastases is evidenced by a persistent positive test after operation

If the directions given are strictly adhered to, negative results are to be expected in less than 2 per cent of normal or pathologic pregnancies when the fetus is still living In nonpregnant individuals or in women more than 4 days after the death or delivery of the fetus and complete removal of the placenta, negative results are secured in over 98 per cent of cases studied The accuracy of the test is even greater if two rabbits are used, since a false negative test will be found in one of the rabbits in about 2 per cent of the cases In ectopic pregnancy, the test is of value only when it is positive, because false negatives occur in about one third of the cases, due to death or degeneration of the chorion

From the above discussion, it is evident that a positive Friedman test indicates the presence of living chorionic tissue, that the intensity of the test varies with the amount of such tissue, and that a negative test will occur following the death or degeneration of all chorionic tissue

¹ Zondek B Gonadotropic Hormone in the Diagnosis of Chorion Epithelioma J A M A 108 607-611 (Feb 20) 1937

² Ferguson R S Pathologic Physiology of Teratoma Testis J A M A 101 1933 (Dec 16) 1933

³ Hinman F and Powell T O Management of Tumor of the Testicle J A M A 110 188-190 (Jan 15) 1938

⁴ Cutler M and Owen S E Clinical Value of Prolan A Determinations in Teratoma Testis Am J Cancer 24 318-325 (June) 1935

⁵ Schoenck F J Quantitative Friedman Test in Hydatidiform Mole and Vomiting of Pregnancy Am J Obst & Gynec 32 104-109 (July) 1936

⁶ Evans H M Kohls Clara L., and Wonder D H Gonadotropic Hormone in the Blood and Urine of Early Pregnancy The Normal Occurrence of Transient Extremely High Levels J A M A 108 287-289 (Jan 23) 1937

A hormone test for the determination of the sex of the unborn child has been proposed, but has not proved reliable

III OTHER LABORATORY TESTS IN PREGNANCY

A *Urinalysis*—A complete routine urinalysis should be done every two weeks after the diagnosis of pregnancy is established, and preferably once a week in the last three months

1 *Reducing Substances in the Urine*—These should always be identified. In the first three months a moderate amount of dextrose in the urine is a common finding due to the lowered renal threshold and need not cause alarm. If found later in pregnancy or if associated with clinical symptoms of diabetes mellitus, dextrose in the urine is an indication for a fasting blood sugar estimation and, if that is under 150 mg, for a dextrose tolerance test, since a true diabetes mellitus may first become manifest during pregnancy. Reduction in the last three months of pregnancy and during lactation will usually be due to lactosuria and when the co-existent excretion of dextrose has been ruled out by a negative dextrosazone test, may be regarded as physiologic

2 *Albuminuria in Pregnancy*—This is a common finding. Most frequently it is due to passive congestion of the kidney from disturbed return flow in the renal veins but it may be due to pyelitis, nephritis or any of the other causes of albuminuria listed in Chapter II, or it may be the first sign of impending eclampsia. It should be regarded as an indication for more frequent urinalysis and blood pressure readings, and a close search should be made for its cause. In eclampsia a four plus albuminuria is the rule, associated with oliguria, casts and sometimes, hematuria. Complete anuria is not uncommon

3 *Ketonuria*—This should be tested for, as the presence of acetone is an indication for increase in the carbohydrate intake. A marked ketosis is the rule in pernicious vomiting of pregnancy and is an indication for performing an alkali reserve estimation and giving dextrose, intravenously if necessary, until the ketosis has disappeared and the alkali reserve is normal

4 *Microscopic Examination of the Urine*—This is very important because of the frequent finding of pus (usually due to pyelitis) and of casts (nephritis, eclampsia, etc). Other sediments have the same significance as in the non pregnant

B *Hematology*—A routine hematologic examination should be made once a month, keeping in mind, however, that a moderate simple leukocytosis which becomes marked during labor and in the

puerperium is physiologic and that a decrease of 15 to 20 per cent in the hemoglobin and red cell count in the latter months is the rule. A greater decrease should be regarded as an indication for increasing liver and iron in the patient's diet. A sudden marked decrease with high color and volume indexes and normal saturation index warrants the diagnosis of pernicious anemia of pregnancy which requires prompt and vigorous treatment. The blood cell sedimentation rate may prove of value in detecting abnormalities in the puerperium.

C Quantitative Blood Tests—These are not necessary if complications do not arise. If vomiting occurs, blood chloride and alkali reserve determinations should be made. If hypertension develops, the blood urea nitrogen, the concentration and dilution test, and the urea clearance estimation are important.

IV TOXEMIAS OF PREGNANCY¹

Notwithstanding the conflicting views expressed in a voluminous literature the following points are probably correct. Pernicious vomiting and eclampsia are definite entities which occur only in association with pregnancy or with hydatidiform mole. Aside from this, their etiology is unknown. The chief pathology of each is found in the liver. Much confusion has arisen in the past from a failure to differentiate from these "toxemias" the various disorders of the kidney and urinary tract discussed in Chapter II which may begin or first be discovered during pregnancy. Only very careful clinical and laboratory study together with accurate definition and use of terminology and a careful follow up of the further course of the patient will serve to make this differentiation. Uremia and eclampsia are not synonymous terms and should never be used interchangeably.

A Pernicious Vomiting—This is a disorder of the first half of pregnancy characterized by persistent nausea and vomiting. Many theories have been advanced as to the etiology, of which one of the most recent² is that it is due to allergy to the hormone produced by the corpus luteum. Since ketonuria develops on an adequate carbohydrate intake and before the nausea and vomiting begin, hypersecretion of ketogenic hormone by the pituitary is a possibility that should

¹ Hurwitz D. Toxemias of Pregnancy. *New England J Med* 209: 1281-1291 (Dec 21) 1933.

Strauss M B. Observations of the Etiology of the Toxemias of Pregnancy. V. The Etiologic Relationship Between Water Retention and Arterial Hypertension. *Am J M Sc* 196: 188-198 (Aug) 1938.

Dieckmann W J. Renal Function in the Toxemias of Pregnancy. *Am J Obst & Gynec* 29: 472-488 (Apr) 1935.

² Finch J W. The Etiology of Nausea and Vomiting of Pregnancy. Preliminary Report. *J A M A* 111: 1368-1370 (Oct 8) 1938.

be investigated. Since the clinical diagnosis is easy,¹ laboratory tests are of value, not for diagnosis, but as guides to therapy and in determining the severity of the patient's condition. The urine should be tested daily or oftener for acetone and diacetic acid, and the 24 hour volume should be recorded. A ketosis is usually present in untreated cases due to the deficient carbohydrate absorption, and is a specific indication for giving dextrose by rectum or vein. Oliguria is an indication for increasing the fluid intake. The alkali reserve estimation should be performed frequently. The reason for this is that ketosis or dehydration, if allowed to become severe, tends to produce acidosis while loss of hydrochloric acid from the stomach tends to produce an alkalosis. Hence, the alkali reserve figure may be high, low or normal, irrespective of the severity of the case, but any deviation from the normal is an indication for controlled therapy. The blood chloride estimation should be done if vomiting is severe, as a low estimation due to loss of hydrochloric acid is a common indication for the administration of sodium chloride. A high urea nitrogen and non-protein nitrogen in the blood will be found in the more severe cases as in all other types of severe vomiting, but should not occur in properly treated cases. The uric acid is increased in the blood as in all cases of starvation but no information of value is secured by its estimation. It will be noted that all of the above changes occur in nausea and vomiting from any cause, they must be regarded, therefore, as results rather than causes of the condition. The only laboratory finding to which etiologic significance may logically be attributed is hypoglycemia, but its existence is disputed. It certainly occurs in some cases, and should be tested for when the patient is first seen, because after dextrose therapy is started the results of blood sugar estimation will be of little diagnostic value. Albuminuria is not infrequent but does not alter the prognosis.

B Eclampsia²—This is a specific toxemia of pregnancy occurring usually in the last three months, during labor, or in the puerperium. The more thoroughly cases thought to belong in this group are studied by competent internists, the fewer remain. Peters³ even doubts the existence of this condition as a clinical entity. Certainly it is a diagnosis which should be made after elimination of the conditions listed

¹ Providing this possibility is considered whenever vomiting is the major symptom in a woman over 10 and under 50 years of age and the Friedman test is used.

² Bell E. T. Renal Lesions in the Toxemias of Pregnancy. *Am J Path* 8: 1-42 (Jan) 1932.

Stander H. J. and Cadden J. F. Blood Chemistry in Preeclampsia and Eclampsia. *Am J Obst & Gynec* 28: 856-871 (Dec) 1934.

³ Peters J. P. The Nature of the Toxemias of Pregnancy. *J A M A* 110: 329-331 (Jan 9) 1938.

under V It seems possible in view of the recent work of Goldblatt that most of the symptoms of eclampsia may be secondary to hypertension resulting from pressure of the enlarged uterus on the ureters and renal blood vessels Many other theories of its etiology have been presented, including a disturbance of water balance, of plasma proteins or placental necrosis Clinically it is characterized by hypertension, coma, convulsions, and oliguria or anuria, none of which findings will differentiate it from uremia At necropsy, the typical liver changes which do not occur in true uremia, make the differentiation easy for the pathologist The only laboratory tests which are of differential diagnostic value are the blood urea nitrogen (or the non protein nitrogen) and the blood creatinine estimation These are high in true uremia but in eclampsia the blood creatinine is normal and the urea nitrogen does not exceed 30 mg The urea clearance is within normal limits unless the urine volume is under 1 cc a minute These tests should always be performed since uremia is almost certain to develop again if other pregnancies are allowed to occur, while eclampsia does not show this tendency The following tests are indicated as guides to therapy and prognosis but are of no differential diagnostic value as similar findings occur in any of the conditions which may lead to uremia The alkali reserve estimation is important as it usually reveals acidosis requiring treatment The urine will be of small volume, of high specific gravity, and will contain three to four plus albumin and casts, with or without red cells The blood sugar level averages above normal The value of this determination is disputed There are extreme fluctuations in the blood sugar level with a very rapid fall (usually from a preceding high level) to a relative or absolute hypoglycemia just before each convulsion with a return to the high level following the convulsion Clinical improvement is reported to follow dextrose therapy

The following changes occur but are largely of research interest, as they are neither of diagnostic nor of prognostic value The uric acid is constantly increased in the blood The proportion of the total nitrogen of the urine occurring in the form of ammonia nitrogen is often over 10 per cent, the increase occurring at the expense of the urea nitrogen fraction Tests of liver function (Chapter V) may be done but so far have yielded little information of value

These cases may be recognized before the onset of convulsions or coma At this stage it is permissible to apply the term pre eclampsia, but the majority of patients previously so classed probably have been cases of "low reserve kidney" of acute or chronic nephritis or of

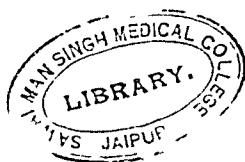
it is not certain that it is the primary etiologic factor and not merely a secondary invader

H Osteomalacia¹—This is a disturbance of calcium and phosphorus metabolism, occurring most frequently in pregnant women. It is characterized clinically by bending and fracture of the bones resulting in great deformity. There is a great deficiency of calcium and phosphorus in the bones and these elements are much decreased in the blood stream. It occurs in persons on a limited diet who are not adequately exposed to sunlight. Hence, it is common in China and India, and a great increase in its frequency occurred in the Central European countries in the starvation period during and immediately following the World War. Its etiology is not definitely determined, but the theory having most support is that it is an adult form of rickets and, therefore, due to a deficiency in vitamin D.

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PART TWO
LABORATORY METHODS



SECTION I USE AND CALIBRATION OF APPARATUS

A Burettes—1 **Choice and Care**—The 25 cc type, graduated in 0.1 cc is most satisfactory for routine laboratory use, but smaller sizes, e g, 5 cc graduated in 0.02 cc, may be obtained for micro methods. Burettes are obtainable either with a glass stopcock or for use with a pinchcock¹ and rubber connection to a glass tip. The all glass type is slightly more accurate, but is less convenient for clinical use because the tip and stopcock are hard to clean, are corroded by alkaline solutions, are easily broken and the stopcock sometimes sticks tight. A further disadvantage is that the bore of the tip is not adjustable. In the other type, several tips of different sized bore may be used on the same burette for delivering small or large drops.

To fill a burette properly, close the pinchcock and pour in the solution, through a small funnel, to a point well above the graduations, force out air bubbles by compressing the rubber tubing above the pinchcock, turn the glass tip up until the point is above the level of the rubber tubing and then open the pinchcock until the air bubble is forced out and solution flows from the tip, adjust the top level to the zero mark.

When through with the burette for the day, drain the contents, rinse with tap water and distilled water, invert to facilitate drainage, and leave the pinchcock on the glass tip and not on the rubber tubing. When using the same burette for different solutions successively, it is important to rinse first with distilled water and then with some of the solution next used.

2 **Calibration**—In order to secure accurate results in any type of quantitative analysis it is absolutely necessary first to recalibrate all measuring apparatus unless it has been certified by the Bureau of Standards or is factory tested with a certificate furnished. The error in volume delivered or contained should not exceed 0.4 per cent.

All glassware should be cleaned with cleaning solution² before calibration.

When calibration by the weight of mercury or of pure distilled water contained or delivered is not possible because of lack of fine balances, it may be done with an accurate burette or pipette certified by the

¹ Some prefer a solid glass bead inserted in the rubber tubing.

² Make this fresh once a week by adding *slowly* 2 parts of concentrated sulphuric acid to 1 part of 20 per cent sodium or potassium dichromate solution.

Bureau of Standards or factory tested with certificate. A certified 2 cc pipette, graduated in 0.02 cc or a 1 cc pipette, graduated in 0.01 cc is very useful for measuring small amounts of solution accurately, as well as for calibration of other apparatus. To calibrate a burette with a certified burette, fill the latter with water to the zero mark and then drain out to the 25 cc mark, allowing proper time for drainage (40 sec for 25 cc), fill the burette to be calibrated to the zero mark with water and then run out 5 cc portions into the certified burette, allowing time (10 sec for 5 cc) for drainage, read the certified burette and make note of any significant error, repeat the process until the entire volume is tested.

To test a burette with a certified pipette, fill to the zero mark, drain to the 25 cc mark, allowing time for drainage, and then measure in 5 cc portions with the certified pipette, noting errors as above.

3. Technic of Titration—In acidimetric work put the standard alkali in the burette. The solution to be titrated should be placed in an Erlenmeyer flask of 300-400 cc capacity and this placed on a plain white background, such as unruled paper or white tile, to facilitate detection of the end point. The indicator is added and the titration is begun, keeping the solution agitated constantly.¹ The solution in the burette may be added somewhat rapidly at first but slowly when the end point is approached. When very near the end point add a fraction of a drop at a time, and mix after each addition. In certain cases it is of advantage to compare with a control or color standard using a white background and reflected light from a source of light behind the operator.

The Erlenmeyer flask is preferable to the beaker and stirring rod because it is much faster, requires less manipulation, and there is no danger of losing the estimation by poking a hole through the container or by splashing out of solution.

B. Pipettes—**1. Types**—There are three types of pipettes for routine laboratory work, the plain, graduated, and volumetric. The plain type is for use when the quantity is unimportant as in qualitative tests or to secure a drop of fluid for microscopic study, and has no graduations. A medicine dropper is superior for most purposes for which plain pipettes are usually used.

The graduated (Mohr) type is for more accurate work and is calibrated in 0.1, 0.05, 0.02, or 0.01 cc from the zero mark to the tip or to the final mark just above the tip. The volumetric type is most accurate, has only one mark and has a dilated portion between the tip and

¹ Right handed persons should learn to manipulate the pinchcock with the left hand and keep the solution swirling in the titration flask at the same time with the right hand.

this mark. This type is intended to measure accurately by drainage and should not be blown out.

Micropipettes for measuring volumes of 0.2 cc or less are often very inaccurate, even if standardized by the Bureau of Standards. Therefore, micromethods should be avoided whenever possible and, where necessary, as in hematology, the pipettes should be recalibrated.¹ This may be done by titrating equal volumes of accurate dilutions of a strong (about 6N) potassium iodate solution made with the pipette to be tested and with a tested 1 or 2 cc pipette and comparing the values obtained. An excess of potassium iodide and N/1 sulphuric acid are added and the solutions are titrated with N/200 sodium thiosulphate, using starch as indicator, as in the Shaffer Hartman sugar method. For use when many such calibrations are to be made, a pipette calibrator has been designed² which operates on the principle of mercury displacement by micrometer screws and will calibrate pipettes of less than 5 cc capacity with great speed and accuracy.

2 Calibration.—All pipettes should be checked for accuracy either by the weight method or against a certified burette as described above. Time for drainage should be determined if not noted on the apparatus. Ostwald pipettes and pipettes marked to the tip must be blown out after proper time of drainage if the full amount is measured. If the zero mark on the volumetric type is not accurate, make a new one on a piece of label and test it. When the correct level is determined a new permanent mark may be made with a file or a diamond tipped glass pencil.

Semi automatic delivery pipettes,³ connected to overhead siphon bottles, are convenient for large laboratories. Burettes or calibrated lengths of glass tubing connected to overhead siphon bottles, with provision for exit of air and exclusion of dirt, aid in rapid measurement of exact quantities.

3 Care.—When through using a pipette rinse it well with distilled water and stand it vertically, with the tip upward, on clean absorbent material such as a paper towel or in a special drying rack to drain and dry.

Before using a pipette for standard solutions or for accurate measuring, it should be dried or else rinsed with a little of the solution to be

¹ Dunn F. I. The Calibration of White Blood Cell Dilution Pipettes. *J. Lab. and Clin. Med.* 19, 95-100 (Oct.) 1933.

² Obtainable from the Shaw Surgical Co. Portland, Oregon.

³ Osgood I. F., Osgood A. H. and West F. S. An Accurate Pipette Calibrator and Microaspirator. *Am. J. Clin. Path. Tech. Supp.* 9, 128-133 (May) 1930.

⁴ Jennison M. W. A Rapid and Accurate Semi Automatic Delivery Pipette. *Am. J. Pub. Health* 24, 59 (Jan.) 1934.

used Never lay a pipette down in such a way that the tip becomes contaminated

C Graduated Cylinders—These are graduated, cylindrical vessels obtainable in various sizes, 25, 50, 100 cc, etc They are not accurate and should be used only for rapid, rough measurement by delivery

D Volumetric Flasks—These are flasks with a long, narrow neck graduated to contain or deliver one exact quantity at a certain temperature It is important to allow the correct time for drainage when using for delivery and to keep in mind the fact that a flask calibrated to contain (marked E, T C, or "to contain") 100 cc will not deliver 100 cc from the same mark because of solution adhering to the glass

E The Microscope—A complete discussion of the mechanics, optics and operation of the microscope is given in the literature accompanying the apparatus only the methods of avoiding common errors and two of the special attachments will be discussed here

1 **Care**—A microscope should be covered with a cloth or a plastic dust cover shaped like a bell jar or put in a special box when not in use The objectives especially the oil immersion lens, should be wiped off with lens paper and xylene or benzene and then dried with lens paper after they have been used All movable parts should occasionally be lubricated with a light grade of machine oil

2 **Use**—North daylight gives the most accurate color values but a microscope lamp with a blue glass window is most convenient and dependable for routine use Adjust the amount of light both with the diaphragm shutter and by the height of the condenser It is important to reduce the light intensity when examining such things as urinary sediment and parasitic ova Always focus the object by raising the objective, i e lower the objective until it almost touches the object with the eyes on a level with the object, then look through the ocular and raise the objective until the object is brought into focus Certain combinations of ocular and objective are best adapted to each kind of work The ordinary microscope is equipped with 5x and 10x oculars and 16 mm, 4 mm, and the 19 mm (oil immersion) objectives An 8 mm objective is well worth the additional cost If the microscope is to be used for laboratory diagnosis only and not for examination of tissues, money may be saved and an even more convenient optical system obtained by substituting an 8 mm objective for both the 4 mm and 16 mm objectives and omitting the 5x eye piece The combination of the 10x eye piece and 8 mm objective gives a magnification of 200x which is ideal for examination of urinary sediments blood searching for intestinal parasites and survey of food or marrow atypical cells to be examined further if the microscope is satisfactory for

notation of this value along with the ocular, objective, and tube length used. Usually it is possible to adjust the tube length so that the factor for converting spaces to micra is an even number.

4 **Dark field Illumination**—This is a form of illumination in which the object appears light and the background dark. In order to be visible, the objects must be in a refracting medium different from themselves and must have either strongly refracting or reflecting qualities. The optical arrangements must be such that the object is lighted by a beam of light which cannot get into the objective either because the rays are so oblique or because they are cut out before reaching the eye. Only that light which is reflected, refracted, or diffracted by the object reaches the eye. The procedures necessary for dark field observation are: a powerful source of light should be arranged so that parallel rays fall on the mirror, the dark field condenser should be raised until its top is flush with the upper surface of the stage and it should be accurately centered, the preparation to be examined should be mounted on a cover slip and this sealed with vaseline or oil, to a slide of proper thickness, a large drop of immersion oil should be placed on the upper face of the condenser and on the cover slip, the slide should then be placed on the stage so that there is oil immersion contact between slide and condenser, the oil immersion objective should be focused on the object and a final adjustment of the light should be made with the mirror. The achromatic oil immersion objectives with built in diaphragm are convenient if the microscope is to be used for both dark field and other purposes.

F **The Colorimeter**—This is an instrument for comparing the intensity of the color of two solutions, not the character of the color, although the results are more accurate when the character of the color is the same in each solution.

1 **Choice**—An instrument of the plunger type having a lamp in the base, such as the Klett or Kober, is the most satisfactory. A colorimeter lamp is better than daylight because the intensity of the light is more constant.

2 **Setting the Instrument**—Make sure that the cups and plungers are clean and dry or rinsed with the standard solution. Put the same solution in both cups, set them in the instrument and make sure that they are in the proper position. Acid hematin solution prepared as for an Osgood Haskins hemoglobin estimation is the most satisfactory material for accurately setting a colorimeter which I have found, as it does not change with changes in temperature and very slight changes in intensity of color transmission are detectable. Raise the cups until the plungers touch the bottom of the cups, the verniers should read exactly zero. If they do not, make sure the cups are properly located and the plungers screwed up tight. If the verniers still do not read zero when the plungers are in contact with the bottom of the cups, adjust the position of the verniers until they do read zero. Then set both sides at the same reading, preferably somewhere in the range of

color intensities that occur in the actual estimation. Now make both sides match by changing the position of the lamp in the base. Check the adjustment by making several readings on the solution in the right hand cup as though it were an unknown.

In matching, start with a *slightly* too pale color and gradually intensify it. Be careful not to tire the eye, looking at the colored light for only a few seconds at a time. If in doubt as to the end point, turn the screw back and forth from the point which is the least detectable degree too pale to the point which is the least detectable degree too intense, and when the extent of this excursion has been mentally noted stop in the mid point of this excursion and take the reading. Eye fatigue errors will be greatly reduced if as little time as possible is consumed in bringing the color intensities to equality. Therefore, in repeated readings, turn the cup adjustment only sufficiently to make a slight difference in color intensity before starting. Constantly ask yourself whether this is deeper or paler, not whether these are the same in character of color.

When the adjustment is correct, securely fasten both the lamp and the colorimeter in position so that they cannot be moved or, if using the lamp in base type, tighten the set screw holding the bulb in position. Place some mark of identification on the left hand cup and use it for the standard in all estimations. This is important because the bottoms of the cups vary in thickness and the zero point may be incorrect if they are interchanged.

To perform an actual estimation, place the standard in the marked cup on the left hand side and set it at the reading specified for the standard in the method used. Place the unknown in the right hand cup and match it with the standard. Take the average of at least three readings. When matching pale colors, more accurate results may be obtained by placing a plate of ground glass between the cups and the source of light. In some determinations accuracy is increased by color filters to limit the wave length of light transmitted to a narrow band at the point of maximum absorption of the compound to be determined.

3 Care of the Colorimeter —When through with the instrument, rinse out the cups and dry them and place in a covered box to keep out dust and prevent breakage. Wash and dry the plungers also. Between estimations of the same type rinse the cup with a little of the unknown solution that is to be used next. When changing standards, the cups and plungers should be rinsed and dried before the addition of the next standard. When the colorimeter is not in use, keep it covered with a cloth or bell-jar to keep out dust and moisture.

G The Biocolorimeter—This may be made from a block of wood according to the specifications given (Fig 9) It should be painted black. With a suitable set of standards, almost any colorimetric method can be adapted for use with this. It is less accurate than the plunger type colorimeter for clear solutions, but more accurate for determinations in which there is interfering turbidity or color. It is well adapted for determinations on serum or urine.

1 Principle—A graded series of clear standards is compared with the unknown in which the specific color has been developed with water

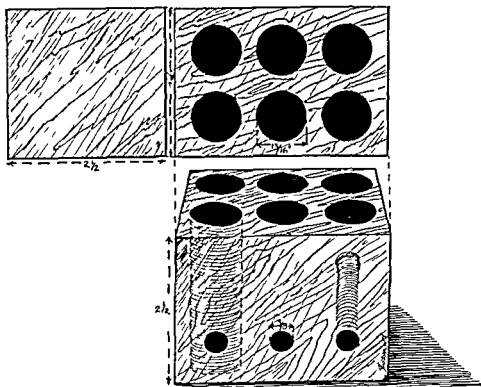


FIG 9—Construction of a Biocolorimeter

behind the unknown and the serum or urine containing the interfering turbidity or color behind the clear standard. It is necessary that all of the test tubes be of the same shape and diameter.

H Photo Electric Colorimeters—A number of photo electric colorimeters are now on the market. They are constantly being improved so they may become obsolete quickly. They do not increase the accuracy of most determinations over that obtainable with a good colorimeter in expert hands but they do considerably increase the rapidity of estimation when many determinations are run at a time. The photo electric colorimeter will probably pay for itself in time saved in any laboratory doing more than 100 colorimetric determina-

tions a day. It should be remembered, however, that photo electric colorimeters introduce sources of error which are less apt to occur in ordinary colorimetry. The photo electric cells detect only light intensity so that differences in character of color or in turbidity are not noticeable as warnings of some error in technic or contamination of a standard. The photo electric cells are so sensitive that a grease mark such as a thumb print on the side of the container may introduce significant error. Mechanical failure of the apparatus is more apt to occur and more difficult to correct than in other types of colorimeters. The accuracy of all subsequent determinations will depend on the accuracy of calibration of the colorimeter for the method and unless frequent checks are used an error in calibration is more likely to remain undetected than in a regular colorimeter. Any colorimetric or turbidimetric method may be adapted for use with the photo electric colorimeter by determining three or four widely different concentrations on known solutions and plotting the results on semilogarithmic graph paper. Directions for use accompany each instrument.

SECTION II PREPARATION OF STANDARD SOLUTIONS¹

A Prerequisites—These are a fine balance that will weigh accurately to 0.1 milligram, measuring apparatus certified by the Bureau of Standards, factory tested with certificate, or recalibrated as described above, a supply of distilled water and chemically pure reagents or approved directions for preparing them

B Standard N/10 Sodium Hydroxide—This is prepared from a stock solution of about 60 per cent² sodium hydroxide (dissolve 100 grams of sticks of pure sodium hydroxide in 125 cc of water) which has been allowed to stand until clear Dilute 4 cc of the stock solution to 500 cc (approximately 15 per cent stronger than N/10) with distilled water and place in a tightly corked non sol or Pyrex flask, or a flask coated on the inside with paraffin This is to prevent corrosion of the glass and alteration of the strength of the solution (dissolving of alkali from the glass)

For checking this solution I prefer an M/10 solution of potassium acid phthalate³ (2.0414 grams of pure crystals dissolved and diluted to 100 cc) because the crystals (free of water of crystallization) can now be obtained chemically pure and ready to weigh⁴ Fill the burette with the sodium hydroxide Measure accurately with a pipette 5 cc of the phthalate solution into a clean 400 cc Erlenmeyer flask containing 10 cc of distilled water, two drops of a 1 per cent alcoholic phenolphthalein solution and enough very dilute sodium hydroxide to give a faintly pink color Titrate to the phenolphthalein end point, observing all the precautions given under the technic of titration above Add another 5 cc of phthalate solution to the mixture and titrate again to see if the two check From the titration figure calculate the dilution of the sodium hydroxide necessary to produce an exact N/10 solution, make the dilution of the entire amount, and test it by titration as above

Example of calculation If 9.8 cc of the sodium hydroxide solution are equivalent to 10 cc of the M/10 phthalate solution then 9.8 cc contain the amount of alkali that is desired for 10 cc therefore every 9.8 cc should be diluted to 10 cc The following proportion may be used for calculating the dilution of this solution

$$9.8 : 10 :: \text{total volume of stronger than N/10 NaOH} : x, \text{ "x" is the volume to}$$

¹ All reagents and standards mentioned in this text may be purchased ready prepared from the Shaw Surgical Co Portland Oregon

² In quantitative chemistry the percentage of a solution refers to the number of grams of the solute contained in 100 cc of the solution

³ A more commonly used method of checking standard alkali (NaOH) is by titration (with phenolphthalein) with exact N/10 oxalic acid prepared by weight from pure crystals The crystals are purified by recrystallization and are then thoroughly air-dried to preserve the proper water of crystallization The objections to this method are the extra manipulations necessary to obtain the pure substance and the possibility of over or under-drying

⁴ When making standard solutions transfer the weighed substance to a volumetric flask partly fill the flask with solvent dissolve and dilute to exact volume never add the crystals directly to the total volume of solvent When making exact dilutions do not mix the smaller quantity and the supplementary volume but transfer the smaller quantity to a volumetric flask and dilute to exact volume

which the strong ($N/10 +$) alkali must be diluted to give an exactly $N/10$ solution. If exactly one liter of solution is desired use the following formula $9.810 \times 1000 \div x$ 'x' is the volume of solution stronger than $N/10$ to dilute to one liter to give an exactly $N/10$ solution.

C Standard $N/10$ Sulphuric Acid—Measure 1.6 cc of C P sulphuric acid into a 500 cc volumetric flask, dilute to the mark with distilled water and mix. This will give a solution about 10 per cent stronger than $N/10$. Titrate 5 cc portions as directed in B with the exact $N/10$ sodium hydroxide¹ (prepared as in B). From the titration figure calculate the dilution of the stronger than $N/10$ acid necessary to produce an exactly $N/10$ sulphuric acid.

D Standard $N/70$ Solutions—Prepare these by exact dilution of the $N/10$ solutions with distilled water. A rapid way is to use a 100 cc and a 500 cc volumetric flask. Measure exactly 100 cc of $N/10$ solution in one flask and 500 cc of distilled water in the other flask. Empty both into a large dry flask or bottle using part of the water to rinse the solution out of the small flask. Take 100 cc more of water with the small flask and empty. Mix well, rinse both flasks with some of the $N/70$ solution and pour it back into the large bottle. Prepare both acid and alkaline solutions and check them by titrating the acid with the alkali. They should check within 0.4 per cent. It is best to measure the acid with the same pipette that is used to measure the acid for an actual estimation.

¹ A method commonly used for checking $N/10$ sulphuric acid is by titration (with methyl orange or sodium alizarin sulphonate not with phenolphthalein) with exactly $N/10$ sodium carbonate prepared by weight from pure dried powder. The latter is obtained by strongly heating C P sodium bicarbonate until sodium carbonate is formed. The dry powder is cooled in a desiccator before weighing. The objections to the method are the extra manipulations necessary to obtain the pure dry powder and the possibility of not completely converting the bicarbonate to carbonate.

SECTION III CHEMISTRY AND MICROSCOPY OF THE URINE¹

A Routine Qualitative Examination—The following tests are usually included in a routine urinalysis the color, appearance, odor, specific gravity, reaction, sulphosalicylic acid test for protein, reduction, acetone, and microscopic examination of the sediment. The reaction and specific gravity could well be omitted on single specimens and be done only if specifically ordered since they give little information of value unless done on accurately collected and properly preserved 24 hour specimens. The first time a reduction test is positive on the urine of a particular patient the reducing substance should be identified. If acetone is present a test for acetoacetic acid should be done. If the sulphosalicylic acid test gives a precipitate which disappears on boiling, the substance responsible should be identified. If the color is deep brown or yellow the test for bile should be done. If the color is smoky red or dark, the test for hemoglobin should be done. If it is purplish red the test for porphyrins should be done. A dark brown or black color indicates the test for alkaptonuria or melanin.

In large laboratories much money may be saved by using smaller quantities for tests than are recommended in most texts. Color or turbidity may be seen as easily in 1 or 2 cc. as in 10 or 20 cc.

Proper planning of the urinalysis will save much time if many specimens are to be examined. First start the heat under the water bath so it will be boiling when it is needed. Arrange the specimens in sequence and number report forms to correspond. Keep these forms in sequence. Test tube racks for the albumin and acetone tests should be permanently numbered as should the racks for the boiling water bath and the positions for the centrifuge tubes. Fill the centrifuge tubes and start the centrifuge. While these urines are centrifugating place the proper amount of Benedict's solution in the test tubes and arrange them in the rack and put about 1 gram of sodium nitroprusside and ammonium sulphate mixture in each tube for the acetone test by dipping the end in the powdered material. Remove the tubes from the centrifuge into numbered racks. With a medicine dropper transfer 0.3 cc. of urine to the Benedict's tubes and about 1 cc. each to the corresponding albumin and acetone tubes. Rinse the dropper in running water and repeat for each successive specimen. Place the Benedict's tubes in the water bath which should be boiling and set the interval timer for five minutes. Decant the supernatant fluid from the centrifuge tubes and do the microscopic examination, recording the results as they are done. Remove the Benedict's tubes from the water bath when the bell rings and allow to stand until the sediments are completed and the

¹ Kilduffe R. A. Clinical Urinalysis and Its Interpretation. Pp 428. F. A. Davis Co. Philadelphia 1937.

acetone and sulphosalicylic acid tests are ready to read. Using a 10 cc pipette with a rubber bulb add ammonia to each of the acetone tubes. An especially built rack holding the tubes inclined at an angle of about 30° in front of the corresponding albumin tube is convenient. Then with the sulphosalicylic acid in a dropping bottle add the proper amount to each albumin tube. Mix and heat the tubes to boiling that show a cloud. Read the results of the albumin, reduction and acetone tests and record them on the slips all at once. Do acetoacetic acid tests on the urines with positive acetone and test for Bence Jones protein on the urines in which a cloud in the sulphosalicylic test clears on boiling. Start the tests for identification of the reducing substance on the urines from patients who have not previously had positive reduction tests. The specific gravity may be noted most efficiently if glass tubing of proper diameter to contain the urinometer is cut off and fitted with a one hole rubber stopper. Support it in a clamp over the sink and attach by means of a short length of glass tubing a rubber tube going down the sink drain. Pour in the urines while pinching the tube or using a pinchcock and note the color, odor, appearance specific gravity and reaction. Allow to drain and pour in the next, recording the results as each is done. Complete the tests indicated and record the results. Using this system an experienced technician can do 100 urinalyses in two and a half hours.

1 Collection and Labelling of Specimens—Under ordinary conditions the composition of the urine varies greatly at different hours of the same day. For this reason the collection of the urine is extremely important and no quantitative test is of much value unless a sample of the mixed 24 hour specimen is used and the total 24 hour volume is accurately known. The patient or the one responsible for the collection of the urine should be given specific instructions preferably written about the time and manner of starting the collection, care after collection, and mixing and measuring of the total volume.

The patient should void at a specified time say 8 A M. and discard this urine, then save *all* the urine voided from then until 8 A M. of the next day at which time the bladder should be completely emptied whether there is a desire to void or not. The urine should be saved in a *clean* bottle or fruit jar to which has previously been added 1 cc of toluol. It is best to keep a supply of large bottles in the laboratory for this purpose. The bottle should be shaken after each new addition of urine. At the end of the 24 hour period the total sample should be well mixed, measured carefully and an 8 ounce portion saved for examination. If only qualitative tests are desired excepting in the case of a diabetic under treatment a single specimen voided at any time of day will serve the purpose. A bottle of 8 ounce capacity with a wide mouth and a tight fitting cap having a place for the date and patient's name is recommended.

Labelling of specimens is extremely important and should be done as soon as the urine is voided. The label should include the patient's name in full, the exact time of collection (minute, hour and day) whether voided or taken by catheter and if in a hospital the floor and ward number. All of this data must be transferred to the permanent laboratory record. A system of forms has been devised¹ which serves as the order form, the label on the specimen, the report form and the record form and saves writing for all persons concerned.

¹ Osgood E. E. A Simple Set of Laboratory Forms. J. Lab. & Clin. Med. 22: 1176-1183 (Aug.) 1937.

Examination for the presence and approximate amount of pus or red cells must be made on a catheterized specimen. If a culture or stain for bacteria is desired the urine must be obtained by catheter and collected in a covered sterile bottle.

2 **Color**—Record this as pale, straw, yellow, amber according to the intensity, if the color is normal. It varies considerably even in health, largely because of variations in the amount of urine voided and variations in the diet. The usual color is due chiefly to variations in the concentration of urochrome. Traces of uroerythrin and urobilinogen are frequently present. Abnormal colors may be orange, green, brown, red, blue, purple, or black and indicate further examination to determine the cause.

A green, or brownish color, especially if the foam is yellow, is an indication to test for bile pigments and bile salts, and a reddish or smoky appearance is an indication for a chemical test for hemoglobin if the microscopic examination for red cells is negative even if these tests were not ordered. Methemoglobinuria gives rise to a brown color associated with brown stained casts and usually positive albumin and occult blood tests. Such findings indicate a spectroscopic examination, a study of the blood for methemoglobin and an interview with the patient to determine what drugs have been taken. Porphyrinuria gives a purplish red color to the urine. Such a color, therefore, is an indication for a porphyrin test and for inquiry as to the ingestion of sulphonal, trional, or tetronal. It may occur as a congenital anomaly. Occult blood tests will be negative. A brown or black color may be due to homogentisic acid (alkaptonuria), melanin¹ (melanosis), or phenol poisoning and is always an indication for saving the urine for special study. Dinitrophenol gives rise to an orange color. A green or blue color results from administration of methyl thionin chloride (methylene blue), a red color, from mercurochrome instillation, and argyrol instillation may explain a brown color.

3 **Appearance**—Record this as clear, slightly turbid, turbid, or cloudy. Freshly voided normal urine is usually clear. Upon standing, mucus and epithelial cells, especially in urine from women, settle and form a faint cloudiness in the bottom of the container. Amorphous urates precipitate in acid urine and form a "brick-dust" or flesh colored sediment which disappears on dilution or warming. Amorphous phosphates precipitate in alkaline urine and form a whitish sediment which grossly may be mistaken for pus but which disappears on acidification with acetic acid. None of these types of cloudiness are abnormal although they frequently alarm patients who happen to observe them.

¹ If on addition of bromine water a yellow precipitate forms which turns brown or black melanuria is probable and the melanin test is indicated.

Red blood cells give the urine a smoky appearance as well as a reddish or brown color. Pus cells in sufficient quantity produce a whitish cloudiness but settle out leaving the urine clear. Bacteria in large numbers produce a uniform cloudiness which does not settle out and cannot be filtered out with filter paper. The record of the microscopic examination should clearly indicate the cause of any turbidity.

4 **Odor**—This should be recorded in accurately descriptive terms (e g, aromatic, ammoniacal, putrid, fruity, etc). Normal urine has a characteristic aromatic odor probably due to aromatic acids. The odor is more marked in concentrated urine. Various articles of diet (asparagus), certain drugs (turpentine), and some of the pathological constituents (acetone) of urine impart a characteristic odor. Decomposed urine has an ammoniacal odor.

5 **Specific Gravity and Total Solids**—The specific gravity may be most conveniently estimated by means of the urinometer, in the tube described on page 339, in a cylinder or in the specimen bottle if of the wide mouthed type. Other methods are with the Westphal balance and with an instrument similar to the hydrometer used for testing storage batteries in which the urine is aspirated into a tube containing the urinometer. With any of these methods the requirements are enough urine to float the urinometer, the float must not touch the sidewall of the container, there must be no bubbles on top of the urine, the container and urinometer must be rinsed between each reading with some of the urine to be used next, and the temperature of the urine must be between 20 and 25° C. The specific gravity, to be of most significance should be taken on a well mixed 24 hour specimen, but preliminary information may be obtained from single specimens.

The grams of total solids excreted in 24 hours may be roughly estimated as follows. Multiply the last two figures of the specific gravity (considered as a whole number) of the mixed 24 hour specimen by 26 and then multiply by the total volume in cc divided by 1000.

6 **Reaction**—This must be taken as soon as possible after the urine is passed and is best determined with Squibb's nitrazine paper comparing with the color chart furnished. This gives an approximate idea of the pH. If both red and blue litmus paper are used, there are four types of reaction: acid, alkaline, neutral, and amphoteric. If acid, both papers are red, if alkaline, both are blue, if neutral, neither changes color, and if amphoteric, both papers change color slightly. To be of most significance, the reaction should be taken on the mixed 24 hour specimen. If the urine is acid to litmus paper and gives -

a red color with 0.05 per cent methyl red in 50 per cent alcohol, it indicates that the urine is sufficiently acid (pH 5.4 or less) for the action of mandelic acid or methenamine (urotropin) in the treatment of urinary infections. If the urine is alkaline to litmus paper and gives a deep red or purple color with 0.1 per cent aqueous solution of phenol red, it indicates that the urine is alkaline enough (pH 7.4 or more) for the treatment of urinary infections with sulfanilamide. If accurate information on the reaction is desired, the pH should be determined with a Beckman model G pH meter or some instrument of similar type. The directions for use of this instrument accompany the apparatus.

7 Protein—The results of this important test should be recorded on a scale of + to + + + +, grading from the least detectable positive test to the strongest that occurs in which the precipitate solidifies in the tube so that it will no longer pour.

The proteins which may occur in the urine are serum albumin, serum globulin, proteose, Bence-Jones protein, and mucin. Occasionally others may be found but they are of no importance, clinically. Serum globulin and serum albumin usually occur together and need not be differentiated from each other. They are usually both understood to be present when the terms heat coagulable protein or "albumin" are used. Urine to be tested for protein must be perfectly clear, therefore, cloudy urine must be filtered or centrifugated.

(a) *The Sulphosalicylic Acid Test*—This is the most satisfactory routine test for protein. It is a very delicate test for all urinary proteins of clinical significance and will differentiate albumin and globulin from proteose and Bence Jones protein. A rough quantitative estimate should be made from the density of the cloudiness. The technic is as follows: to about 1 cc. of clear urine add 4 drops of a 20 per cent sulphosalicylic acid solution. If no cloudiness appears protein is absent. If a cloudiness appears, heat the mixture to boiling and then cool under the tap. If the cloudiness persists on boiling and remains on cooling, albumin or globulin, or both, are present. If the cloudiness disappears on boiling and reappears on cooling, either mucinous protein, proteose, Bence-Jones protein, resin acids, urates, or bile salts are present and further tests are indicated. If both Bence-Jones protein and albumin or globulin are present, the cloudiness does not completely clear on boiling, but usually becomes less dense. Do not confuse a clumping together of the precipitate and adherence to the walls of the tube with a true clearing by solution.

¹ Osgood E. E., Harkins H. D. and Wilhelm Mable M. Tests for Protein in Urine Especially Bence Jones. J. Lab. & Clin. Med. 16: 575-582 (March) 1931.

(b) *Further tests* to be done if a cloud in the sulphosalicylic acid test clears on boiling —(1) Salt and acetic acid heat coagulation test — To 5 cc of clear urine in a test tube add 1 cc of 50 per cent acetic acid and if no precipitate appears, add 3 cc of saturated sodium chloride solution and mix. Heat the mixture to boiling. A cloudiness appearing in the cold on the addition of acetic acid is usually due to bile salts, resins, or mucinous protein. A cloudiness appearing in the cold on the addition of the saturated sodium chloride solution suggests the presence of Bence Jones protein but may be due to a large amount of globulin. If a cloudiness appears on boiling and remains on cooling, albumin or globulin, or both, are present. If a cloudiness appears in the cold which clears on boiling and reappears on cooling, Bence Jones protein is probably present and further tests for this substance are indicated. If both Bence-Jones protein and albumin or globulin are present a cloudiness will appear in the cold which becomes heavier at about 60° C but does not completely clear on boiling. The great advantage of this technic over the technic commonly used is that the precipitation in the cold immediately attracts attention to the possibility that Bence Jones protein is present, thus leading one to test further for this rare but important substance.

(2) Hydrochloric acid ring test — Carefully overlay the urine on 1 or 2 cc of C P hydrochloric acid. A ring occurs if Bence Jones protein, resin acids or bile salts are present.

(3) Bence Jones protein ¹—If the preceding tests are positive this test is necessary to prove conclusively the presence of Bence Jones protein. It is generally stated that Bence Jones protein precipitates on warming the urine to 60° C and redissolves on boiling to reappear again on cooling. This statement is true only if the reaction of the urine and its salt content are just right. If such a result is obtained it is good evidence that Bence Jones protein is present but experience has shown that if the urine is not exactly right as regards reaction for its salt concentration, the protein may fail to precipitate at any temperature or may precipitate and fail to clear on boiling. Hence, one should proceed as follows. Warm about 5 cc of urine in a test tube by holding it in a beaker of water the temperature of which is gradually raised. The temperature of the urine should be watched by the aid of a thermometer dipped in the urine itself. Stir frequently. Note the temperature at which the first definite cloud appears and the temperature of maximum cloudiness. Then remove the tube from the bath, dry its exterior and boil for one or two minutes over the direct flame. If the precipitate clears up completely, cool and note the temperature at which it reappears. If it does not clear up add 50 per cent acetic acid a drop at a time continuing the boiling until 1 cc has been added. If the precipitate has not redissolved with this amount it will not redissolve at all and consists in part at least of globulin or albumin. In this case the boiling mixture should be filtered through a *hot funnel*. A precipitate

¹ See reference on p. 342

appearing in the filtrate on cooling and redissolving on boiling proves the presence of Bence Jones protein. If on the first warming no precipitate appears at any temperature, the urine is probably too acid or too dilute and this should be corrected by adding small portions of 10 per cent sodium hydroxide or saturated sodium chloride solution, repeating the gradual warming after each addition. A cloudiness appearing at a temperature of 48° to 65° C, disappearing on boiling, and reappearing on cooling at 65° to 85° C can be safely interpreted as due to Bence Jones protein. The biuret test given below will demonstrate that the precipitate is protein.

(4) **Biuret test**—This is a conclusive test for protein. To 5 cc of urine in a centrifuge tube add 1 drop of hydrochloric acid and 5 cc of 2 per cent phosphotungstic acid solution and mix. Centrifuge, pour out the clear liquid. If necessary, filter instead of using the centrifuge. Mix the precipitate with 5 cc of alcohol, and centrifuge again. Pour out the alcohol. To the precipitate add 1 cc of 10 per cent sodium hydroxide and shake a little. Add 0.5 per cent copper sulphate solution a drop at a time, watching for the characteristic violet color which one should learn by trying the test on known protein solutions. If very little protein is present the color may be seen only in the middle zone. A pure blue color is negative.

(c) **Other Tests in Common Use**—The nitric acid ring test is more expensive and less reliable than Robert's test. Ring tests are not recommended, however, since they do not give any differentiation between albumin, proteose, Bence Jones protein, resin acids, urates or bile salts, and the density of the ring depends more on the amount of mixing than on the quantity of protein present. They are not as simple as the sulphosalicylic acid test. Robert's reagent is made by adding 100 cc of C P nitric acid to 500 cc of saturated magnesium sulphate solution.

The heat and acetic test as ordinarily performed has several disadvantages. Occasionally false positive and false negative results are secured. Most cases of Bence Jones proteinuria will be missed. It requires much more time than the sulphosalicylic acid test.

8 Acetone Test (Rothera)—All urines should be tested routinely for acetone. Dip a one half inch test tube into a mixture of 1 gram of sodium nitroprusside and 100 grams of ammonium sulphate thoroughly ground together in a mortar so as to collect about a one half inch layer of crystals. Add about 1 cc of urine and shake until saturated. Not all the crystals should dissolve. Add 1 or 2 cc of C P ammonium hydroxide and mix. On standing for ten minutes or less a reddish purple color develops, varying from a slight pink to a deep permanganate color depending on the amount of acetone present. If acetoacetic acid is also present it intensifies the test. A deep permanganate color developing at once is a 4 plus reaction. A faint pink appearing only after minutes is a 1 plus reaction.

If the test is negative, it will not be necessary to test for acetoacetic acid since the latter is never present when acetone is absent.

9 Aceto-acetic (Diacetic) Acid—This test should be done whenever the acetone test is positive.

(a) *Lange's Test*—Acidify 1 cc of urine with 1 drop of 50 per cent acetic acid and add 5 drops of 5 per cent sodium nitroprusside solution. Allow 1 or 2 cc of C P ammonium hydroxide to flow down the wall of the tube to form a top layer. Within 1 minute a reddish violet ring will form if aceto acetic acid is present. Disregard the color appearing on longer standing as it is probably due to acetone. A brownish red ring may result from excess of acetone alone but as much as 1.5 cc of pure acetone per liter of normal urine gives a negative test.

(b) *Gerhardt's Test*—This is an older but less delicate test. To 3 to 5 cc of urine add 10 per cent ferric chloride solution by drops until no more precipitate forms. A brownish red color appearing on addition of more ferric chloride to the filtrate is positive. Many drugs, including salicylates, phenol, antipyrin and alkalies that are excreted into the urine give a color reaction also. Boiling the urine to half volume before addition of the ferric chloride will result in a negative test if the color was due to aceto acetic acid. A positive reaction to this test indicates severe ketosis.

10 **Reduction**—All urines should be tested routinely for the presence of reducing substances. The first time a urine from a particular patient shows reduction further tests are indicated to determine if the substance is a sugar and, if so, which sugar it is. To be clinically significant, the urine must contain enough reducing substance to reduce Benedict's reagent in the heating time specified.

(a) *Benedict's Qualitative Test*—If more than one plus protein is present, remove it by the sulphosalicylic acid test and filtration or centrifugation. Use enough of the filtrate to be equivalent to 0.3 cc of urine.

Technic to 5 cc of Benedict's qualitative reagent add 0.3 cc (8 drops) of urine and mix. Heat in a boiling water bath for exactly 5 minutes. (If only one urine is to be tested it may be boiled over a flame for 2 minutes but gently shake the mixture constantly to prevent bumping.) A red, orange, or yellow *finely granular* precipitate which rapidly settles indicates reduction. If only a small amount of reduction is present, allow the tube to stand or centrifugate it before judging the result. Reduction is accompanied by a change of color of the mixture to a green or yellow. After settling the intensity of blue color of the supernatant fluid bears an inverse relationship to the amount of reduction. With complete reduction it becomes almost colorless. The character of the precipitate is the most important criterion. A flocculent precipitate which does not settle rapidly is due to other substances than sugar.

A + reaction corresponds to less than 0.5 per cent dextrose
 A + + reaction corresponds to 0.5 to 1.0 per cent dextrose
 A + + + reaction corresponds to 1.0 to 2.0 per cent dextrose
 A + + + + reaction corresponds to over 2.0 per cent dextrose
 The appearance of these should be learned on dextrose solutions of known concentration

Benedict's qualitative reagent Dissolve 100 gm of sodium carbonate (monohydrated) and 173 gm of sodium citrate in 600 cc of distilled water with the aid of heat. Filter while hot and dilute to 850 cc. To this add, while stirring or shaking, 150 cc of a solution containing 17.3 gm C.P. copper sulphate crystals (i.e., an 11.5 per cent solution).

(b) **Fehling's Test**—This test is unsatisfactory. It will not detect less than 0.1 per cent of dextrose. The reagent is reduced by chloroform, moderate amounts of mucin, uric acid, creatinin, and many other substances often present in urine.

11 Identification of Reducing Substances¹—This should be done on the first urine, obtained from a particular patient, which reduces Benedict's solution. On subsequent urines and urines which do not give reduction tests, these tests need not be done.

(a) **Osazone Test**—This is the most reliable test for determining the kind of sugar present, although it is rather long and does not give a reaction in urine with all kinds of sugar. The technic is as follows: To 3 cc of urine in a test tube add 0.1 gram of pure phenylhydrazine hydrochloride and 0.2 gram of sodium acetate and mix. Heat in a boiling water bath. At 10 or 15 minute intervals remove a drop of the mixed solution and examine it under the microscope for the characteristic crystals. Continue the heating for one hour if crystals do not appear sooner. The sugars found in urine which produce osazone crystals are dextrose, levulose, lactose, maltose, and pentose. Dextrose and levulose produce identical crystals (dextrosazone) and therefore, other tests must be used to differentiate them. Lactose or pentose in urine seldom yield osazone crystals (see Fig. 10).

(b) **Fermentation Test**—This gives reliable results if carried out in the following way on urine to which no preservative has been added: boil 12 to 15 cc of the urine to kill bacteria, cool, and mix with a little yeast (one twentieth of a fresh Fleischman's moist yeast cake) so as to get a good suspension. Pour it into a fermentation tube and remove air from the closed limb. Let it stand for one day at room temperature or at 37° C for an hour or so. Gas in the closed limb of the tube indicates the presence of dextrose or levulose. If no gas is formed, lactose, pentose, or glycuronates may have been responsible for the reduction. Two controls run simultaneously with the unknown are necessary to make the results conclusive, one with boiled normal urine to which lactose has been added which should be negative and one with boiled normal urine to which dextrose has been added which should be positive. The chief objection is that the urine must contain no preservative.

¹ White, F. D. and Green, A. C. A Critical Comparison of Color Tests for Fructose, Pentose and Glycuronates in Urine. Trans. Roy. Soc. Canada Section V, pp. 145-157, 1937.

(c) *Lactose Tests*—This need be tested for only in the urine of pregnant or lactating women or in the urine of infants. The urine should be diluted until it contains less than 10 per cent as indicated by a + or ++ reduction of Benedict's reagent, before any of these tests are done.

(1) A modification of Rubner's test is recommended. Mix 3 cc of urine, 2 cc of C P ammonium hydroxide and 3 drops of 10 per cent sodium hydroxide. Heat in a beaker of boiling water and observe after 2, 3, 4 and 5 minutes heating. A reddish brown color is positive. Heating too long diminishes the color and makes it more brownish. Other sugars give a yellow color. Lactose below 0.2 per cent

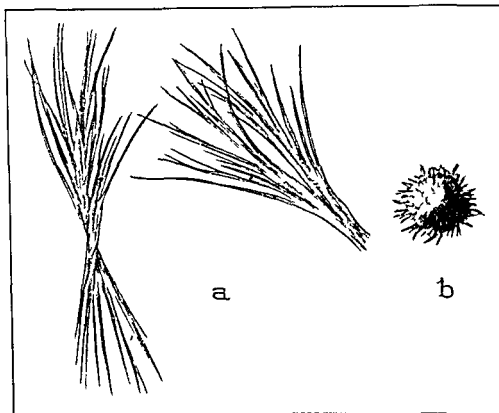


FIG. 10.—Osazone crystals (yellow) a—phenyldextrosazone b—phenyllactosazone

gives too slight a color for detection. Do not remove protein since sulphosalicylic acid interferes with the test.

(2) *Cole's test*. The urine should contain less than 1 per cent of sugar. Charcoal removes from the urine by adsorption all reducing substances except dextrose, levulose, pentose and formaldehyde (from urotropin in acid urine). Lactose in concentrations of less than 1 per cent is also removed. Therefore, it is a valuable test for occasional use to determine that reduction is due to glycuronic acid or lactose.

Shake 0.5 gm. of best adsorbent charcoal (Merck's blood charcoal) with 12 cc. of urine, boil 1 minute, cool and shake frequently during 10 minutes. Filter through a small filter paper. Add 5 drops of 50 per cent acetic acid to the filtrate and boil 1 minute (to decompose urotropin if present). Use 1 cc. for a Benedict's

test (reduction indicates dextrose, levulose, or pentose) If positive, do the osazone levulose and pentose tests on the rest of the filtrate

(d) *Levulose Test* (Seliwanoff) —To 2 cc. of Seliwanoff's reagent add 0.3 cc. of urine and heat in a boiling water bath for five minutes. A deep reddish color which tends to remain distinct even after considerable dilution and is soluble in amyl alcohol denotes the presence of levulose. A dextrose solution of 2 per cent or over will give a reddish color, which, however, fades rapidly on dilution. Therefore, the urine should be diluted before doing the test if the Benedict's qualitative test indicates the presence of over 1.5 per cent of sugar.

Seliwanoff's reagent. Dissolve 50 mg. of resorcin in 70 cc. of water and add 30 cc. of C P hydrochloric acid.

(e) *Pentose Tests* —L xylo ketose,¹ the pentose usually excreted, will reduce Benedict's qualitative solution in the cold, if allowed to stand for 3 hours. Levulose is the only other reducing substance, which may be present in the urine, which will give this test.

Anilin acetate test for pentose (White and Green). To 2 cc. of urine add 2 cc. of glacial acetic acid and 5 drops of pure redistilled anilin. Heat just to boiling, allow to stand for 2 minutes, cool, and extract with chloroform. A red color indicates pentose in a concentration greater than 0.1 per cent. Dextrose gives a green color which decreases the sensitivity of the test, so that if dextrose is present (dextrosazone crystals obtained in the phenylhydrazine test) it should be removed by fermentation with yeast before the pentose test is performed.

Bial's test. To 2 cc. of Bial's reagent add 0.5 cc. (10 drops) of urine and heat until greenish. Add about 1 cc. of amyl alcohol and shake a little (dilution with water will cause the alcohol to separate), the top layer of alcohol will have a pure green color if pentose is present, an olive green is not a positive test. Glycuronates sometimes give positive pentose tests but they can be eliminated by treating the urine with charcoal (Cole's test).

Bial's reagent. Dissolve 3.0 gm. of pure orcinol in 100 cc. of 95 per cent alcohol and add 2.0 cc. of 10 per cent ferric chloride. This solution keeps. As used, add 1 part of this to 9 parts of C P hydrochloric acid.

(f) *Glycuronates* —These may cause the urine to give positive reduction and pentose tests but after the urine is treated with charcoal, all these tests are negative.

TABLE 17.—DIFFERENTIATION OF REDUCING SUBSTANCES

	Dex trose	Levu lose	Lac tose	Pen tose	Glycu ronates	HCHO (urotropin)	Homogen ous acid
Benedict's	+	+	+	+	+	+	+
Dextrosazone	+	+	—	—	—	—	—
Seliwanoff's	—	+	—	—	—	—	—
Lactose test	—	—	+	—	—	—	—
Bial's	—	—	—	+	+	—	—
Adsorption (to carbon)	—	—	+	—	+	—	—?
Fermentation	+	+	—	—	—	—	—
Spontaneous oxidation	—	—	—	—	—	—	—

¹ Lasker, Margaret and Enklewitz, M. A Simple Method for the Detection of Xyloketose in Urine. J. Biol. C. 294 (June) 1933

Glycuronates may be detected with the polariscope (levo rotation), if protein levulose and beta hydroxybutyric acid are absent

(g) *Homogentisic acid* (rare) —This is easily detected because the urine turns brown and finally black on exposure to air. It reduces Benedict's reagent

12 Examination of Sediment —This should be done as a routine on every urine. It is best done on the sediment of the entire night sample, collected between the hours of 9 00 P M and 7 00 A M. It should be done as soon as possible after the specimens are collected as many important sediments may dissolve on standing and crystalline sediments of little significance may appear.

If only sediments are to be looked for the urine specimen should be collected in a bottle which has been rinsed out with 10 per cent formalin as this preserves sediments better than other antiseptics.

(a) *Gross Inspection* —A more accurate quantitative idea of the amount and kind of sediment present is obtained if the volume of sediment in the centrifuge tube and its appearance are compared with the results of the microscopic examination. All sediments appear white except uric acid and red cells which are red, amorphous urates which are flesh colored, and indigo which is blue.

(b) *Microscopic Examination* —Transfer 10 cc of mixed urine to a conical centrifuge tube¹ and centrifugate. Use the clear supernatant urine obtained for the qualitative tests and decant the remainder. Note the volume of sediment and mix with the last few drops of urine by tapping the tube with the finger.

Place a drop on a slide and examine with the low power or preferably an 8 mm lens under reduced light. A roughly quantitative estimation of the amount of each kind of sediment present may be made from the number of cells or crystals in a field and the amount of sediment in the centrifuge tube.

(1) *Casts* Figure 11. These are the most important of the urinary sediments. They are albuminous plugs that have formed in the uriniferous tubules and, therefore, are of various sizes and shapes but always have parallel sides and one rounded end. Report the actual number of casts if less than 10 are found in the whole drop of centrifugated urine. Report less than one cast per field as 1+, one to 10 per field as 2+, 10 to 20 per field as 3+, and a packed field as 4+.

(2) *Pus cells (leukocytes)* Figure 11. These are of little clinical significance, particularly in female patients, unless found in catheterized specimens. They have a somewhat granular appearance and show as nuclei after acetic acid is added. Report pus cells in

¹ Always balance such a tube on the scales against another tube.

the centrifugated specimen as occasional if there are less than one per 8 mm field, 1 to 20 per field as 1+, 20 to 50 as 2+, over 50 as 3+, and a packed field with grossly purulent urine as 4+

The three glass test is often of value in determining the approximate source of pus and blood in the urine of male patients. The patient should be instructed to empty the bladder, collecting the urine in three

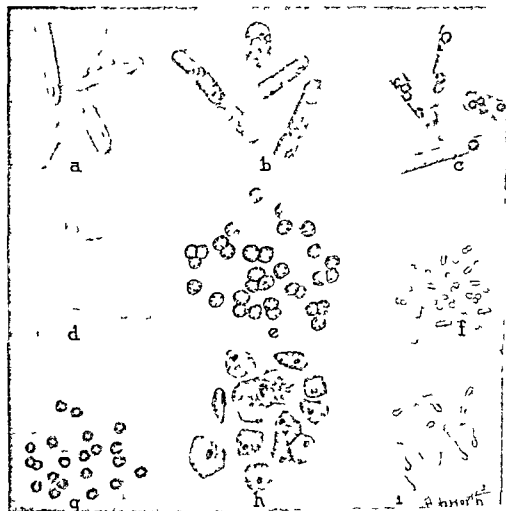


FIG 11—Organic urinary sediments $\times 200$ (a) Hyaline casts (b) Granular casts (c) Red cell casts (d) Cylindroids and mucus threads (e) Pus cells (f) Bacteria (g) Red cells (h) Epithelium (i) Spermatozoa

successive containers. The flow of urine must not be stopped between glasses. The relative amounts of shreds, pus, and blood should be noted by gross and microscopic examination and the results recorded for each glass. The following things may produce confusing results: large amounts of phosphates may produce a cloud when the urine is voided (clear with acetic), blood clots, large amounts of pus, etc., may

settle in the bladder, and appear only in the third glass, leaving a clear urine for the first and second glass

(3) Red blood cells Figure 11 These are of little clinical significance in female patients, unless found in catheterized specimens They appear as very small, round objects having a greenish tinge with the low power and a yellowish tinge with the high power They disappear on the addition of acetic acid On increasing the light intensity, pus cells disappear from view while red cells still are visible If there is any doubt about the identification of red cells, a smear of the sediment should be dried, stained with Wright's stain and examined with the oil immersion lens Chemical blood tests on the sediment are not satisfactory for this purpose The result of the three glass test has the same significance and limitations as in pyuria

For cells in the centrifugated drop report less than 10 cells per 8 mm field as occasional, 10 to 30 cells as 1+, over 30 cells per field as 2+ if no visible blood is seen in the centrifuge tube, a packed field with a visible layer of red cells in the sediment in the centrifuge tube as 3+, and with a grossly bloody urine as 4+ Remember that a very small amount of blood will produce a large number of red cells in the sediment, and a few drops of blood will color a liter of urine, so that the tendency is always to over estimate the amount of blood lost in this manner As small an amount of blood as 0.2 cc in a liter of urine will show a packed field if 10 cc of the mixed urine is centrifugated and the sediment is examined

(4) Epithelial cells Figure 11 These are identified by the size, shape, and the presence of a round nucleus They are larger than pus cells These are of several types, depending upon their source in the urinary system (contamination from the vagina must be excluded)

(5) Other organized elements Cylindroids (Fig 11) may be confused with hyaline casts by a beginner, but they are stringy not hyaline in structure and the sides are not parallel but taper to a slender tail which is usually twisted or bifurcated The only importance of recognizing them is to avoid reporting them as casts

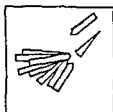
Spermatozoa (Fig 11) are readily recognized from their characteristic structure, using the 4 mm objective and a dim light

Bacteria and other micro organisms may be detected with the 4 mm objective but are best identified by staining a fixed smear of the sediment with the proper stain They are of importance only if present in urine obtained with a catheter with aseptic precautions or if found in very large numbers immediately after the urine is voided

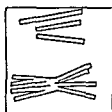
The ova of *Schistosoma haematobium* are often found in the urine of patients in Northern Africa or less commonly in the Southern United States. Hematuria is usually associated.



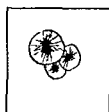
Triple Phosphate



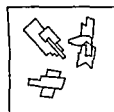
Calcium Phosphate



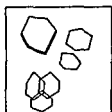
Calcium Sulphate



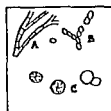
Calcium Carbonate



Cholesterol

A Leucine - (Yellow)
B Tyrosine

Cystine

A Magnesium Phosphate
B Indigo (Blue)A Mold
B Yeast
C Starch

Calcium Oxalate

Uric Acid
(Yellow)Sodium Urate
(Yellow)Ammonium Urate
(Yellow)

URINARY SEDIMENTS

FIG 12

(6) Contaminations which get into the urine after or while being passed include yeast cells, moulds, cotton and wool fibers, oil globules, starch, hair, cork and other vegetable cells and also epithelial cells, bacteria, pus, or blood from the vagina.

(7) Chemical sediments (Figure 12) —Acid urine Those likely to be found in acid urine are yellow, brown or red crystals of uric acid, yellow or green sodium urates, and colorless calcium oxalate, acid calcium phosphate, calcium sulphate, or cystin The last three sediments are rarely seen Amorphous urates appear as dustlike particles under the microscope but are salmon pink or flesh colored as seen in the specimen bottle or centrifuge tube

Alkaline urine Those likely to be found in alkaline urine are crystals of ammonium magnesium phosphate (triple phosphate), acid calcium phosphate, brown or yellow ammonium urate, normal magnesium phosphate, calcium carbonate, or of indigo The last three sediments are rare The common amorphous phosphates appear as a white sediment in the centrifuge tube and structureless dustlike particles under the microscope

The following rare sediments may occur in urine of any reaction cholesterol crystals, yellow leucin associated with tyrosin, and fat globules which may be identified by their orange staining with Sudan III Contamination with petrolatum or other oils must be excluded before concluding that fat droplets were present in the urine as voided In lipoid nephrosis doubly refracting lipoid droplets may be found in the urine (identified by the polarizing microscope)

The ability to identify urine sediments correctly is very important It can be acquired only by much controlled practice on known sediments Looking at plates will refresh one's memory on sediments previously seen, but this is not a satisfactory way of learning the microscopy of urine If difficulty is encountered in identifying crystalline sediments they should be tested chemically, as directed for urinary calculi

(c) *Quantitative Sediment Count* (Addis¹) —After the usual breakfast in the morning the patient must take no fluids or juicy fruits until the collection is complete The patient should not void after 4 P M until 6 or 8 P M The patient should then void, discarding this specimen and noting the time All urine voided after this until exactly 12 hours have elapsed should be collected in a specimen bottle which has been rinsed out with a little formalin In women the urine should be obtained by catheter

Technic —Measure the volume of the 12 hour specimen accurately Warm to dissolve urates or add acetic acid in just sufficient quantity to dissolve amorphous phosphates if such sediments are present Mix

¹Addis T A Clinical Classification of Bright's Disease J A M A 85 163-167 (July 18) 1925

thoroughly Transfer exactly 10 cc to the Addis graduated centrifuge tube with narrow tip, obtainable from Arthur H. Thomas and Company Centrifugate for 5 minutes at 1800 revolutions per minute Decant most of the supernatant fluid and remove the rest with a pipette or medicine dropper, leaving a volume slightly greater than the volume of sediment and not less than 0.5 cc Note this volume, mix the sediment thoroughly, and using a fine pipette make a mount on each of the two sides of a hemocytometer After allowing time for settling, count all the casts, leukocytes and red cells seen in the entire ruled area If only a few are seen, make several mounts, and if many are present make a dilution such that countable numbers are obtained Determine the average number over the ruled area which is equal to 0.9 c. mm Divide this by 9 and multiply by 10,000 to get the number in 1 cc of the mixed sediment Multiply this by the volume in which the sediment was suspended to get the number in 10 cc of urine since all of the sediment from 10 cc of urine is concentrated in this volume Multiply this figure by 0.1 of the measured 12 hour volume to get the number of casts or cells excreted in a 12 hour period

Hines¹ has devised a nomograph which simplifies the calculation which is obtainable from J. W. Stacey, Inc., San Francisco Report the number of casts, red cells and leukocytes excreted in the 12 hour period Normal values are given on page 25

13 Chemical Identification of Urinary Calculi and Crystalline Sediments—

These vary in size and shape as well as in composition They are of two types, the simple, made up of but a single constituent, and the compound, made up of two or more constituents The compound calculi are by far the most common Most calculi consist of an arrangement of the material in concentric rings about a central nucleus The nucleus may be uric acid crystals, calcium oxalate phosphate, clotted blood clumps of bacteria, foreign bodies, etc In the chemical examination the most valuable data are obtained by analyzing each of the concentric layers separately The calculi must be crushed and powdered and divided into several small portions before beginning the chemical examination

EXAMINATION OF CALCULI

(Heat some of the powder on platinum foil)

Does not burn (mostly inorganic)

Burns (mostly organic)

A Treat with HCl CO₂ gas CaCO₃

A Without giving a flame

B Gently heat cool and add HCl CO₂ gas

x Gives murexid test (powder + a few drops HNO₃ and evaporate red residue)

CALCIUM OXALATE

C Treat with KOH and heat NH₃ evolved

If positive, add KOH to powder

TRIPLE PHOSPHATES

¹ Hines D. C. A Nomograph for Simplifying Computation of the Urine Sediment Count (Addis) Am J M. Sc. 187 841-844 (June) 1934

D Treat with HNO_3 dissolves add ammonium molybdate and heat yellow precipitate **PHOSPHATES**

(a) NH_3 evolved **AMMONIUM URATE**

(b) No NH_3 evolved **URIC ACID**

2 No murexid test, dissolves in HNO_3 without CO_2 gas evaporation on water bath yellow residue add alkali orange then heat red **XANTHIN**

B With a pale blue flame and sharp odor dissolves in ammonia characteristic crystals on spontaneous evaporation **CYSTIN**

If there is doubt about the identification of crystals from their morphology they may be tested with 50 per cent acetic acid C P hydrochloric acid and ammonium hydroxide Add a drop of the reagent to a drop of sediment on a slide and observe the effect under a microscope Acetic acid dissolves calcium and magnesium phosphates triple phosphates and calcium carbonate (carbon dioxide is given off) Hydrochloric acid dissolves all crystals except uric acid and calcium sulphate Ammonium hydroxide dissolves cystin and tyrosin These same tests may be applied to fragments of calculi Cystin does not give the murexide test, which differentiates it from uric acid The insolubility of calcium sulphate in hydrochloric acid differentiates it from calcium phosphate The insolubility of calcium oxalate in acetic acid differentiates it from calcium carbonate

14 Tyrosin Test—A test for the presence of tyrosin in the urine has been suggested as a test of liver function The technic is as follows If protein is present it must first be removed by heat coagulation and filtration To 2 cc of the protein free urine add an equal volume of Millon's reagent The solution turns red if tyrosin or any other substance containing the phenol ring is present in considerable amounts

Millon's reagent Under a hood or in a flask attached through a two hole rubber stopper to a suction pump digest 25 grams of mercury with 36 cc of C P nitric acid When all of the mercury is dissolved, dilute the solution to 100 cc with water

A quantitative test has been devised¹ but is much more difficult than this qualitative procedure

15 Cystinuria²—Cystinuria may occur without separation of crystals If Brand's test is positive confirm the presence of cystin by Sullivan's test which is more specific

Brand's test Mix 5 cc of protein free urine and 2 cc of freshly prepared 5 per cent sodium cyanide (poison!) After 10 minutes add 0.3 cc of 5 per cent sodium nitroprusside solution A permanganate color similar to that in the acetone test is positive A brown color is negative and excludes cystinuria

Sullivan's test Proceed as in Brand's test but instead of nitroprusside, add 1 cc of freshly prepared 0.5 per cent 1,2 naphthoquinone 4 sodium sulphonate mix and at once add 5 cc of 10 per cent anhydrous sodium sulphate in N/2 sodium hydroxide After 30 minutes add 1 cc of 2 per cent sodium hydrosulphite in N/2 sodium hydroxide A red color is specific for cystin or cysteine

¹Lichtman S S and Sobotka H An Enzymatic Method for the Detection and Estimation of Tyrosine in Urine J Biol Chem 85 261 (Dec) 1929

²Lewis H B The Occurrence of Cystinuria in Healthy Young Men and Women Ann Int Med 6 183-192 (Aug) 1932

16 **Melanuria** ¹—A positive test is diagnostic of melanocarcinoma, but a negative test does not exclude this diagnosis. Evaporate the 24 hour urine sample to one fourth volume. Add 1 gm of potassium persulphate for each 100 cc. After 2 hours add an equal volume of absolute methyl alcohol. After the precipitate has settled, decant the supernatant liquid and collect the precipitate on a filter paper. Wash successively with water, methyl alcohol, and ether. If melanin is present, a brownish black precipitate remains on the paper which is soluble in 5 per cent sodium hydroxide and reprecipitates on acidification.

17 **Porphyryn** (Garrod) —Add 20 cc of 10 per cent sodium hydroxide to 100 cc of urine which precipitates the phosphates and carries the porphyrin down with it. Filter and dissolve the precipitate by pouring through the filter 20 cc of a warm 10 per cent solution of hydrochloric acid in alcohol. The characteristic absorption spectrum of acid porphyrin with 3 bands: one at 597 millimicrons in the yellow, one at 553 millimicrons in the green, and one at 410 millimicrons in the purple is diagnostic. It should give a deep red fluorescence with ultra violet light, also.

18 **Stains and Cultures for Bacteria** —These should be made on all aseptically collected specimens which contain pus.

19 **Bile Tests** —Either bile pigments or bile salts or both may be excreted into the urine so it is necessary to test for both.

(a) **Bile Pigments** (bilirubin rarely biliverdin) —Both the foam and Gmelin Rosenbach tests should be applied if the color of the urine suggests the presence of bile or if there are clinical indications.

(1) **Foam test** Considerable quantities of bile pigments give a yellow color to the foam when the urine is shaken vigorously. A negative test does not exclude the presence of small quantities of bilirubin.

(2) **Gmelin Rosenbach adsorption test** Acidify the urine slightly with hydrochloric acid and filter as much as possible through a small filter paper. Hang up the paper and, when air dried, test it with drops of yellow nitric acid. Around each drop appear rings of color, red, violet, blue, and green if bile pigment is present in appreciable quantities.

(3) **Huppert Cole test** This test should be done if the Gmelin Rosenbach test gives a doubtful result or if it gives a negative result and it is desired to detect a trace of bilirubin if it is present. The test is performed as follows. Mix 7 cc of urine and 1 drop of saturated magnesium sulphate in a centrifuge tube and add drops of 5 per cent barium chloride as long as it causes a precipitate. Mix well and centrifugate. Decant the liquid. Add to the sediment 3 cc of alcohol, 1 drop of C P sulphuric acid, 1 drop of 5 per cent potassium chlorate solution and stir with a rod. Heat the tube in a boiling bath 1 minute. The alcohol acquires a green or blue color if bile pigments are present. The colored substance is soluble in chloroform; decant the liquid into a test tube, add 1 cc of chloroform and 5 cc of water and shake.

¹Blackberg S N and Wanger Justine O. *Melanuria*. J A M A 100 334-336 (Feb 4) 1933.

²Or test with diazo reagent and alcohol as directed under the van den Bergh test on blood serum. Hunter Geo. A. *Diazo Method for Detecting Bilirubin in Urine*. Canad Med Assn J 23 823-824, 1930.

(b) *Bile Salts* —These tests are extremely satisfactory for detecting the addition of bile to urine but in clinical jaundice they are usually negative even when much bile pigment¹ is present. They are not specific for bile salts.

(1) *Surface tension test* Sprinkle a little finely powdered sulphur called flowers of sulphur, on the urine. The presence of bile salts in a concentration of 0.01 per cent or more or of anything else lowering the surface tension to a similar degree will cause the sulphur to sink rapidly. Settling after agitation is inconclusive. The du Nouy tensiometer will give information on the surface tension which is of research accuracy.

(2) *Pettenkofer's test* This is the most satisfactory test for bile salts in urine but is not absolutely specific. Mix 1 or 2 drops of urine in an evaporating dish with a few grains of cane sugar and 2 drops of concentrated sulphuric acid and warm gradually. A pinkish to permanganate color appears in the mixture if bile salts are present but is difficult to differentiate from the colors that may result from interaction of the acid with other substances sometimes present in the urine. A control without the sugar should show distinctly less color if the test is positive. The test is sometimes faint or negative even when the urine contains much bile pigment. The character of the color should be learned by testing urine to which bile has been added.

20 *Urobilinogen* —Add 0.2 cc of reagent to 2 cc of a well mixed 1 to 10 dilution of *freshly voided* urine. If after standing 10 minutes at room temperature a red tint is visible on looking down through the liquid at a white surface, the test is positive and then should be repeated on 1 to 20, 1 to 30, 1 to 40 etc dilutions until the highest dilution is found in which the test is still positive. This is the one to be reported. Never warm the mixture. Formaldehyde, as after urotropin, is said to interfere with the test and so do nitrites which may be formed in infected urine.

Urobilinogen reagent Dissolve 1 gram of dimethylaminobenzaldehyde in a mixture of 40 cc of C P hydrochloric acid and 60 cc of water. The reagent does not keep indefinitely.

21 *Blood Tests* —The finding of red blood cells in the microscopic examination of the sediment is the most conclusive test for blood as such. If there is any doubt about the identification of red blood cells in the sediment, a Wright's stain (p. 477) should be done on a smear of this sediment. If the urine is distinctly reddish or brownish with no red blood cells present a chemical test for hemoglobin should be made, and if positive the form of hemoglobin present should be identified whether clinical indications for these tests have been recognized to exist or not.

¹ A possible explanation for this is the following. Bile salts are formed at a relatively slow rate and are normally conserved by absorption from the intestinal tract and re excretion in the bile. Hence the rate of excretion in the bile gives an entirely false impression of the rate of formation. If obstruction occurs the circulation of the bile salts is interrupted and they may accumulate in the bile proximal to the obstruction and finally in the blood. Only when the slow rate of new formation has raised the threshold in the blood above the renal threshold for bile salts (as yet undetermined) do they appear in the urine. Hence a strongly positive test for bile salts in the urine would suggest that bile itself had been added to the urine.

(a) *Chemical Tests for Blood*—This is actually a test for hemoglobin (1) The orthotolidin test This test is most satisfactory To 5 cc of urine in a clean centrifuge tube add 1 cc of 50 per cent acetic acid, mix and boil Cool and extract with 3 cc of ether, centrifugating for a few seconds if necessary to secure separation of the two layers Withdraw the aqueous layer by inserting a pipette to the bottom of the tube and aspirating until a little ether enters the pipette and then discard its contents Decant the ether into a clean test tube and if enough blood was not present to give a visible brownish tinge of acid hematin to the ether concentrate to 1 cc under the hot water tap or in a hot water bath keeping the tube at least 6 feet away from the nearest flame Cool and add 0.5 cc of 1 per cent orthotolidin in glacial acetic acid and 1 cc of hydrogen peroxide The orthotolidin solution keeps at least a month A green or bluish green color is positive for blood If the boiling is omitted, the test is only slightly less specific for blood A control test on distilled water which should be negative and another on urine to which blood has been added which should be positive are desirable from time to time as a check on the reliability of the reagents and cleaning process used for the glassware

(2) Benzidin or guaiac tests These are more frequently done, but as the solutions have to be freshly prepared each day, they are much less convenient The technic is the same as given above with the exception that a 2.2 per cent solution of benzidin in glacial acetic acid or a freshly prepared (without heating) alcoholic extract of powdered guaiac is substituted for the orthotolidin and a blue color develops if the test is positive The benzidin tablets on the market are convenient but less reliable and more expensive

The ether extraction is often omitted, but a considerable number of substances other than blood will give positive tests if this precaution is not observed, and small amounts of blood are easily missed

A positive chemical test for blood will be given not only by oxyhemoglobin and reduced hemoglobin, but also by methemoglobin, carbon monoxide hemoglobin or acid hematin which may occasionally be found in urine Hematoporphyrin does not give a positive chemical test for blood

(b) *Identification of Hemoglobin and Hemoglobin Derivatives*—If red cells are not found and a positive chemical blood test is secured, or if the urine is unexplainably red, brown or very dark colored, these substances ought to be looked for whether clinical indications have been detected or not Carbon monoxide hemoglobin, methemoglobin and hematoporphyrin are rarely present in urine and then usually in small amounts, so that their identification is sometimes difficult The color of the urine or the patient's history (drugs) may be the only indication for testing for them They may be most accurately identified with the aid of a good spectroscope by their characteristic absorption bands

22 *Indican*—To 3 cc of urine add 3 cc of Obermayer's reagent and about 1 cc of chloroform Shake occasionally After a time the chloroform becomes blue (indigo) if indican is present The presence of urotropin interferes with the test

Obermayer's reagent Dissolve 1 gram of ferric chloride in 500 cc of C P hydrochloric acid

23 *Diazo Test*—It has been known for a long time that some urines give an orange or red color in an alkaline solution of diazotized sulphanilic acid Ehrlich was the first to apply the reaction to urine but his procedure has been modified many times The original technic was as follows Place equal parts, 1 or 2 cc, of the

diazo reagent¹ and freshly voided urine in a narrow test tube and mix, quickly add 1 cc of strong ammonium hydroxide and shake. Two types of reaction may occur. Type A, given by all urines, is the appearance of a faint yellow color which gradually intensifies for 3 or 4 minutes to a pale orange. Type B, given only by pathologic urines, is the immediate appearance of a brilliant red or bright orange red color (the foam is red also) which disappears in a few seconds. For a more detailed study of the reaction and interpretation of the results see the references.²

24 Arsenic Test—The Reinsch test is the best known and the simplest test but is less sensitive than some other tests. The technic is as follows. Add to some urine in a test tube about one fifth its volume of concentrated hydrochloric acid and a few crystals of C P arsenic free ferrous sulphate. Introduce a piece of arsenic free bright copper foil about 3 mm square and boil for several minutes or heat almost to boiling and let stand for 8 hours. If arsenic be present, a dark bluish gray film is deposited on the copper. Dry the copper in alcohol and ether and insert the rolled copper foil in a 20 cm length of glass tubing. Heat the end of the tubing containing the foil over a flame. Arsenic, antimony and mercury sublime and condense on the walls of the tube. Bismuth does not sublime. Examine the walls of the tube under the low power of a microscope. Arsenic appears as octahedral crystals.

25 Mercury Test—The Reinsch test may be used in the same manner as for arsenic except that the urine is heated only to 60° and then set aside for at least 12 hours. If mercury be present it is deposited on the foil as a bright lustrous mirror and after heating appears on the walls of the tube as fine droplets. Instead of heating a strip of filter paper moistened with cuprous iodide may be placed in a small test tube with the copper foil and after corking allowed to stand for an hour. A pink color develops on the filter paper if mercury is present.

26 Chlorides—This test is of value only in those conditions in which the urine chloride concentration is decreased.

Technic Acidify a few cc of urine with C P nitric acid and add a little 1 per cent silver nitrate solution. Normal amounts of chloride produce a dense white precipitate. In pneumonia only an opalescence may appear.

B Quantitative Methods Including Certain Functional Tests
All quantitative determinations, to be of any value, must be done on accurately collected, labelled and properly preserved 24 hour specimens or fractions thereof.

1 Protein—Ordinarily the information obtainable from properly conducted qualitative tests will suffice for routine clinical work but occasionally a more accurate estimation of the amount of albumin present is desired. Although the error may be as great as plus or

¹ Diazo reagent. Solution A. Dissolve 10 gm of sulphanilic acid in a mixture of 100 cc of C P hydrochloric acid and 100 cc of distilled water (Keeps well). Solution B. Dissolve 0.5 gm of sodium nitrite in 100 cc of distilled water (Make fresh every 3 or 4 weeks).

Make fresh reagent each day by mixing 50 parts of Solution A with 1 part of Solution B.

² Hunter George. The Diazo Reaction in Urine. *Bioch J* 19 No 1 25-33 1925.
Harrison G A and Bromfield R J. The Causes of Andrewes' Diazo Test for Renal Inefficiency. *Bioch J* 22 No 1 43-45 1928.

minus 50 per cent, the technic of the Esbach determination is retained in this edition because it is so widely used, but it is recommended that the method given on page 519 for quantitative estimation of protein in cerebrospinal fluid be applied to a suitable dilution of urine since it is far more accurate and just as simple as the numerous quantitative methods that have been suggested for protein in urine

(a) *Original Esbach Estimation*—The urine should be free of sediment and acid in reaction. Fill the Esbach tube with urine to the U mark, add the Esbach reagent to the R mark, insert a rubber stopper and mix gently. Support the tube in a vertical position. Observe it after one hour and, if precipitate is floating, tap the tube to dislodge air bubbles or gently mix it again. Let it stand for 24 hours and then read on the scale the height of the sediment. The reading is in grams of protein per liter and not in per cent. If a larger amount of protein is present the urine must be diluted with water and then the reading is multiplied by the dilution factor.

(b) *Modification of the Esbach Test*—The Esbach estimation may be made much more quickly in the following manner. Add 0.3 gm of fullers' earth to the urine reagent mixture in the special tube, insert a rubber stopper and mix thoroughly by shaking. Support the tube in a vertical position and read the scale at the end of one hour. For readings below 3.5 there are correction factors, thus between 3 and 3.5 deduct 0.3, between 2 and 3 deduct 0.5, between 1.5 and 2 deduct 0.8, below 1.5 deduct 1.0.

Esbach reagent. Dissolve 5 grams of picric acid and 10 grams of citric acid in 500 cc of water.

2 *Ratio of Night to Day Volume of Urine*—*Technic*—The patient voids at 8 A M and discards the urine, all the urine excreted between 8 A M and 8 P M is measured for the day sample and from 8 P M to 8 A M for the night sample. The ratio between the two volumes is then determined. The only precautions necessary are that the total volume of urine should be over 500 cc, that all the fluid be taken in the daytime, and that an excess of protein be avoided.

3 *Modified Mosenthal Test*—Mosenthal,¹ following the work of Hedinger and Schlayer,² developed the test and established standards for comparison. He recommended a diet with definite contents of chloride and nitrogen but later concluded that an ordinary full diet

¹ Mosenthal H O. Renal Function as Measured by the Elimination of Fluids, Sal and Nitrogen and the Specific Gravity of the Urine. Arch Int Med 16 733-774 (Nov 1915)

² Hedinger M and Schlayer. Ueber die Prüfung der Nierentätigkeit durch Probemahlzeit. Deutsch Arch f klin Med 144 120-166 1914

gave equally satisfactory results. The chloride and total nitrogen determinations on each sample require an excessive amount of time and do not add much of diagnostic value to the information obtained from the specific gravity variations and the ratio of the day to night volume.

(a) *Principle*—Healthy kidneys excrete urine which varies widely in volume, specific gravity, and concentration of salt and nitrogen at different periods within the twenty four hours. Kidneys with decreased functional capacity lose the power to vary these factors and therefore they tend to become fixed near the midpoint and the night volume increases compared with the day volume. The chlorides and total nitrogen may also be determined on each specimen if desired.

(b) *Technic*—The patient remains on the usual diet and fluid intake and daily routine, but takes no liquid at night, that is, after 6 P M. on the day of the test. He should avoid excessive protein or salt intake. At 8 A M. the patient is to empty his bladder and discard the urine. Seven fractions are then collected as follows (voiding punctually at the hour specified and saving all of the urine): 8 to 10 A M., 10 A M. to 12, 12 to 2 P M., 2 to 4 P M., 4 to 6 P M., 6 to 8 P M., and 8 P M. to 8 A M. Instruct the patient to label each sample or supply him with labelled bottles. The volume and specific gravity of each sample is determined as well as the ratio of night to total day volume. The chloride (page 371) and nitrogen (page 367) levels of each sample may be determined but this is now seldom done.

4 **Dilution and Concentration Test**—Volhard has suggested two simple tests which are among the most valuable and sensitive tests available for detecting slight grades of impairment of renal function of the chronic types. These tests should not be done if the patient is edematous or if the blood urea nitrogen is above 50 mg.

(a) *Principle*—The first change that occurs in chronic impairment of renal function is the inability to concentrate and later the inability to dilute the urine.

(b) *Technic*—At 8 A M. the patient is given 1500 cc. of water on an empty stomach. The bladder is immediately emptied and the urine discarded, then urine is collected at one half hour intervals for four hours. The volume and specific gravity of each sample is determined.

Normal results: the total output should be between 1200 and 1800 cc. and the specific gravity should reach 1.003 in at least one sample.

At 8 A M. on the next day, the patient is placed on a diet of solid foods with no fluid intake for 24 hours. The bladder is emptied at the start of the test and the urine discarded, then the urine is collected every 3 hours until 8 P M. and the night urine as one sample from 8 P M. to

8 A M The volume and specific gravity are determined on each sample The specific gravity should reach 1.030 in at least one sample The concentrated urine can to advantage be examined microscopically for casts and red blood cells as recommended by Addis¹

5 Phenolsulphonphthalein Test (Rowntree and Geraghty)

(a) *Principle*—One cc of solution containing 6 mg is injected intramuscularly or intravenously and the per cent of the dye excreted by the kidneys in the next two hours is determined by comparison with a standard This dye is eliminated rapidly and chiefly by the kidneys

(b) *Technic*—Have the patient drink 300 to 400 cc of water 20 minutes before starting the test to promote excretion It is stated that not less than 2 hours should elapse after a meal before injecting the phenolsulphonphthalein The test should not be done within 5 hours after the administration of a saline cathartic

Draw up 1 cc of solution which contains 6 mg of phenolsulphonphthalein into a small sterile syringe and inject into a vein or into the muscles of the back, note the time The patient must empty his bladder immediately Save this urine The intravenous method² is recommended If the intravenous technic is used, collect the first specimen at the end of 30 minutes and the second specimen at the end of 1 hour If the intramuscular technic is used collect the first specimen 70 minutes after injection, and 60 minutes later secure a second specimen Be sure the bladder is emptied each time, use the catheter if in doubt

Standard solution With the syringe measure 0.5 cc of phenol sulphonphthalein solution into a 500 cc graduate, add 5 cc of 10 per cent sodium hydroxide and dilute to 500 cc This solution corresponds to 100 per cent phenolsulphonphthalein excretion

To each of the urines add 5 cc of 10 per cent sodium hydroxide and transfer to 500 cc graduates The estimation may be made with the plunger type colorimeter (method 1), or with the biocolorimeter (method 2)

¹ Addis T The Number of Formed Elements in the Urinary Sediment of Normal Individuals J Clin Invest 2 409-421 (June) 1926 See also page 353

² Rowntree L G and Geraghty J T An Experimental and Clinical Study of the Functional Activity of the Kidneys by Means of Phenolsulphonphthalein J Pharm and Exp Ther 1 579-666 (June) 1910

³ If the intravenous technic is used the ureters may be catheterized by an expert urologist and the urine collected separately in flasks or test tubes containing 1 cc of 10 per cent sodium hydroxide Note the time interval between the injection and the first appearance of the dye (pink color) and determine the proportion of the dye excreted by each kidney in a 15 minute 30 minute or 1 hour period using the same technic as for the intramuscular test The intravenous technic may also be used in cases in which the ureters are not catheterized in which case the urine is collected at 30 minutes and 1 hour after the injection and the total for each 30 minute period and for the whole hour is reported

Method 1 Dilute each urine gradually, comparing the color with that of the standard so that the red of the urine mixture shall not be much paler than that of the standard. The second urine may not allow dilution beyond 100 cc. If the urines contain much pigment it is desirable to use a 50 per cent standard made by mixing equal volumes of the regular standard and normal urine from the first voiding. Filter the diluted urines if they become turbid after adding the alkali. Set the standard at 10 mm (or the 50 per cent standard at 20 mm) make several readings with each urine.

$$\text{Calculation Per cent excreted} = \frac{10}{\text{mm unknown}} \times 100$$

This is on the basis of dilution of the urine to 1 liter, correct for other dilutions as follows: if the volume was 500 cc divide the result by 2, if 250 cc by 4, if 200 cc by 5, if 100 cc by 10.

Method 2 Dilute each urine to about 200 cc and add 5 cc of 10 per cent sodium hydroxide, then dilute to 1 liter, or, if the color is very weak, dilute to 250 or 500 cc dividing the observed results by 4 or 2. After mixing filter a few cc of each urine mixture. To make the estimation, fill the vial furnished with the standards secured from Hynson, Westcott, Dunning Co., place it in the middle hole of the comparator, and in the other holes put the standards that most nearly match the unknown. The per cent is read directly from the ampoule containing the standard.

A biocolorimeter may be made (Fig 9), a set of standards prepared by dilution of the 100 per cent standard, and these standards sealed in test tubes of uniform diameter. The standards retain their color value for about a year if they are kept in the dark when not in use. Between 5 and 50 per cent the standards should correspond to 5 per cent intervals and above this to 10 per cent intervals. This method is most practical for clinical purposes.

Note If blood is present in the urine samples, remove it by the following procedure. Directly after adding 5 cc of 10 per cent sodium hydroxide to the urine, add 10 cc of half saturated magnesium chloride solution, heat almost to boiling while stirring. Cool, dilute to the desired volume and filter. Estimate by method 1 or 2. The phenol sulphonphthalein does not adsorb to the precipitate.

6 Nitrogen of Ammonium Salts —(a) *Principle* —The ammonia of ammonium salts is converted into ammonium carbonate by the mass action of the carbonate reagent. The oxalate in the reagent precipitates calcium and magnesium and prevents the binding of ammonia by precipitation of triple phosphates. Ammonia

is then liberated by a rapid air stream, aided by a warm bath, and is carried over into a measured amount of standard acid, forming ammonium sulphate. The acid not combined with the ammonia is then titrated with standard alkali.

(b) *Technic*—Measure the volume of the properly preserved urine sample accurately. Measure into the urine tube exactly 5 cc of urine, add 2 drops of *caprylic alcohol* and about 6 cc of the potassium carbonate oxalate reagent. Attach the tube to the apparatus

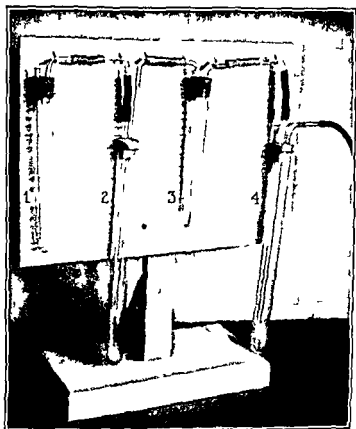


FIG. 13.—Tube No. 1 (8 by 1 inch) 10 cc of 5 per cent sulphuric acid and one drop of methyl orange. Tube No. 2 (12 by 1 inch) urine (or blood) mixture. Tube No. 3 (8 by 1 inch) safety tube (empty). Tube No. 4 (12 by 1 inch) $N/70$ sulphuric acid.

(Fig. 13) at once. Into the last tube measure exactly 25 cc of $N/70$ acid, add 2 drops of *caprylic alcohol* and 1 drop of 1 per cent *sodium alizarin sulphonate*. Attach the tube and connect with the suction pump. Bubble air at a moderate rate for 5 minutes, then use a swift air stream for 30 minutes, immersing the urine tube in a bath of warm water at about 50°C at the start. If one is busy, the aeration may continue for a longer time.

Loosen the stopper of the acid tube and raise the bubbling tube above the liquid. When the bubbling tube has drained, empty the test

tube into a wide mouth 500 cc Erlenmeyer flask. Rinse the bubbling tube, inside and outside with a little distilled water, into the test-tube. Pour the water into the flask and rinse the test-tube again with a few cc of water. Measure into the acid tube exactly 5 cc of N/70 acid and run the air stream for at least ten minutes more. While this is running, titrate the acid in the beaker with N/70 sodium hydroxide to the end point described under the total nitrogen estimation. Finally transfer (with rinsing) the 5 cc of acid to a flask and titrate it.

Calculation Deduct the total N/70 alkali used for both titrations from the total N/70 acid (30 cc). Multiply the cc of acid neutralized (by the ammonia) by the total volume of the urine (100 if percentage is desired) and divide by 5 (cc of urine used), and finally multiply by 0.0002¹. The result is the grams of nitrogen of ammonium salts in the total urine.

Note Control estimation The reagent must be tested for ammonia by running a control. For this purpose, repeat the procedure described above with the exception of using 5 cc of distilled water instead of urine. Deduct the cc of N/70 sodium hydroxide used for titration from 25, this is the control. Test each new batch of reagent that is prepared. If the control is more than 0.1 cc, deduct it from the acid neutralized in each ammonia estimation before making the calculation.

(c) *Apparatus*—An aeration apparatus has been devised by means of which air can be bubbled through a series of tubes (see Fig 13). For large laboratories a more convenient set up can be made by fastening the spring clip type of tool holders obtainable at 5 and 10 cent stores 4 inches apart into the edge of a shelf about a foot above the desk. The tubes are clasped by the clips but can be removed easily for cleaning. As many outfits as desired may be set up in series. The sulphuric acid in tube No. 1 purifies the incoming air, as long as the methyl orange is red the acid need not be changed. The safety tube (No. 3) is to catch the liquid and save the estimation if the urine mixture foams over. Tube No. 4 is connected to a suction pump by rubber tubing or tube No. 1 may be connected to a compressed air vent if the air pressure is regulated carefully.

(d) *Reagent*—Potassium carbonate oxalate reagent. Dissolve 100 grams of pure (U.S.P. is satisfactory) potassium carbonate in 90 cc of distilled water and boil for 5 minutes. Cool, add 10 cc of saturated neutral potassium oxalate solution (30 per cent) and dilute to 140 cc. Generally this reagent is free of ammonia.

7 Nitrogen of Urea—The Van Slyke and Cullen² modification of Folin's ammonia method combined with Marshall's urease method is recommended.

¹ One liter of a normal solution of ammonia contains 14 grams of nitrogen therefore 1 cc of N/70 ammonia contains 0.0002 grams of nitrogen.

² Van Slyke D. D. and Cullen G. E. A Permanent Preparation of Urease and Its Use in the Determination of Urea. J Biol Chem 19 211-228 1914.

(a) *Principle*—Urease, an enzyme obtained from Jack beans completely and quickly hydrolyzes urea to ammonium carbonate. This is neutralized by the buffer phosphate present. From this point on the principle is the same as for the nitrogen of ammonium salts.

(b) *Technic*—Use the same aeration apparatus as for the estimation of nitrogen of ammonium salts. The urine tube and its bubbling tube must be free of alkali¹ before the urine mixture is added and should show a neutral reaction to sodium alizarin sulphonate. Into this clean tube measure 2 cc of an exact 1 to 10 dilution of the urine (equals 0.2 cc), add 1 cc of 10 per cent urease solution,² 4 drops of caprylic alcohol, and 3 cc of 0.6 per cent acid potassium phosphate solution if the urine is acid, or 5 cc if it is neutral or alkaline. Attach the tube to the apparatus and immerse it in a bath of water which is at 55° C at the start (a higher temperature may injure the enzyme). Let it stand at least 15 minutes, allow longer time if possible. For urines containing dextrose 30 minutes must be allowed.

Measure exactly 25 cc of N/70 sulphuric acid into the last tube of the apparatus, add 4 drops of caprylic alcohol and one drop of 1 per cent sodium alizarin sulphonate. When the time for enzyme action has elapsed, connect the apparatus with the suction pump and run the air stream for one minute. Now disconnect the urine tube, but do not remove it from the apparatus, and add about 6 cc of potassium carbonate oxalate reagent. Continue the method from here exactly as in the ammonia estimation.

A control estimation of ammonia in the urease and reagents must be made following out the technic without using urine. Deduct this control in each estimation of urea. Each new batch of urease that is purchased must be tested.

Calculation Subtract the total cc of N/70 sodium hydroxide used for titration from the total N/70 sulphuric acid taken, then deduct the

¹ After each estimation rinse the urine tube and its bubbling tube with tap water then with a small amount of dilute acid and finally with distilled water. Then add 10 cc of distilled water and one drop of sodium alizarin sulphonate (should have an amber color) immerse the bubbling tube and insert the rubber stopper. If after rinsing with this solution the color changes to a yellow or purple continue the rinsing and testing until no color change occurs.

² Prepare the urease solution each day that it is used. If a single estimation is to be made take 0.1 gram of urease and mix in a mortar with 1 cc of water add the phosphate and then pour it into the urine tube. Tablets of 0.1 gram are obtainable crush them before using.

Large laboratories will find it more convenient to use instead 0.1 cc per estimation of the following solution: to 15 gm permutit in a flask add 50 cc of 2 per cent acetic acid and shake 15 minutes. Decant the supernatant liquid and wash the permutit by decantation 3 times with 50 cc of distilled water. Add 30 gm of Jack bean meal or 10 gm of Arlco Urease and 50 cc of distilled water and 0.5 cc of N/10 sulphuric acid and shake gently for 1 hour. Finally add 150 cc of C P glycerol mix well and filter through a creased filter paper. This solution keeps perfectly for 18 months. Use 0.1 cc of this per estimation.

control This volume of acid neutralized by ammonia that has come from the urine must now be multiplied by the factor 0.001 (i.e., 5×0.0002 gm) and by the total volume of urine (100 to get per cent) This figure includes nitrogen of ammonia as well as nitrogen of urea therefore, deduct the amount of nitrogen of ammonia in the corresponding volume of urine to get the nitrogen of urea Grams of nitrogen may be converted into grams of urea by multiplying by 2.143 In urea nitrogen estimations for determination of the urea clearance it is not necessary to deduct the ammonia nitrogen since the ammonia nitrogen in the urine is all formed from urea in the kidney

If only a urea estimation is desired, ammonia may be removed by permittit, dispensing with the necessity for an ammonia estimation Dilute 1 cc of urine to 50 cc with water in a volumetric flask Add one drop of 10 per cent acetic acid if the urine is alkaline before diluting to the mark Mix and pour on to 2 grams of dry permittit in an Erlenmeyer flask Agitate for 5 minutes and filter Use 10 cc of the filtrate for the urea estimation The calculation is the same as given above, except that no deduction for urine ammonia is necessary

The permittit may be reactivated and used over and over again Collect it in a bottle and when a large amount has accumulated free it from ammonia by washing with 10 per cent sodium hydroxide for 15 minutes decanting the supernatant fluid after settling Wash with 2 per cent acetic acid and then with water Dry in the air (not with heat) Test by running an ammonia estimation on 25 cc of a filtrate prepared as above There should be no ammonia indicated if the reagent is satisfactory

8 Micro Kjeldahl Method for Total Nitrogen—Perchloric acid digestion methods are not recommended since there is danger of serious explosion

*(a) Titration Method*¹

(1) *Principle*—The various nitrogenous bodies of the urine are converted into ammonium sulphate when boiled with sulphuric acid and a catalyst The sodium sulphate added raises the boiling point of the solution The ammonium sulphate formed is converted by sodium hydroxide into sodium sulphate and ammonia The ammonia is volatilized by heat in the presence of an excess of alkali and is collected in a standard acid solution The standard acid not combined with the ammonia is titrated with a standard alkali

(2) *Technic*—Set up the apparatus as shown in Fig. 14, then disconnect the rubber stopper with the trap and bent tube from the Pyrex test tube and the condenser Before starting the quantitative determination of total nitrogen ammonia nitrogen or urea nitrogen measure the urine volume accurately

¹Haskins H. D. The Technic of Quantitative Estimation of Urea Ammonia and Total Nitrogen in the Urine Northwest Medicine 18 37-42 (March) 1919

Measure accurately 2 cc of a 1-10 dilution (0.2 cc) of the urine into a *dry* Pyrex test tube (8 by 1 inch), add 1 cc of sodium sulphate sulphuric acid reagent and 2 glass beads¹ to prevent bumping. Support the tube in an inclined position by means of a clamp on a ring stand and insert a bent calcium chloride tube which fits loosely into the Pyrex tube as a fume sucker and attach it to a suction pump.

Heat the tube using a micro burner held in the hand. When the mixture foams above the lower one third of the tube, lessen the heat, otherwise keep up vigorous boiling. When the liquid becomes clear and colorless diminish the heat so that boiling is barely maintained for

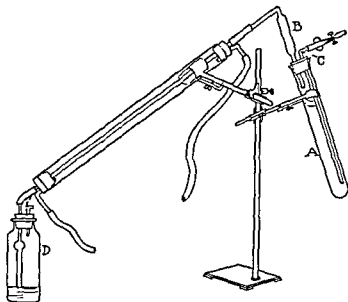


FIG. 14.—Total nitrogen apparatus. A, Pyrex tube; B, safety trap; C, alkali tube; D, receiving bottle.

4 minutes. Ammonia is driven off if the sulphuric acid is boiled down to a small volume by the use of too much heat. If the beads fly above the liquid, tapping the tube will bring them down. Do not allow acid fumes to escape into the air at any time as they might affect the titration later.

In determinations that require longer heating than usual, take away the flame when foaming has ceased, and after the mixture has cooled somewhat, add 3 drops of *hydrogen peroxide*, mix and heat.

While the tube is cooling measure exactly 25 cc of *N/70 sulphuric acid* into the receiving bottle and add 1 drop of 1 per cent *sodium alizarin sulphate*.

¹ A 15 cm. length of Pyrex glass tubing bent into an elongated U and inverted with the open ends under the surface of the liquid is even more efficient in preventing bumping.

Let the tube cool for 2 minutes, keeping the fume sucker attached, then add about 7 cc of distilled water, cautiously at first and with shaking. If the residue is not all dissolved, shake until it is, warming slightly if necessary. The liquid must not be more than luke warm when the strong alkali is added. Adjust the tube in the clamp of the distilling apparatus as shown in Fig 14. The rubber stopper and its attached bent tube and trap is detached from the condenser, and at least 3 cc of 60 per cent sodium hydroxide is drawn up by slow suction beyond the bend of the tube and is held in by clamping the rubber tubing with a pinchcock. After connecting with the condenser allow the alkali to flow down and mix. Adjust the delivery tube to touch the surface of the acid. Heat gently at first and then boil vigorously but watch for bumping since alkali may go over if the liquid is thrown up into the trap. Boil down to about one half volume, or until salts separate out. If it is boiled down to a very small volume, the salts may cake on the glass and the tube is likely to crack.

Disconnect the condenser from the safety bulb, and raise the delivery tube in the receiving bottle. With a spray of distilled water from a wash bottle rinse the inside of the condenser tube and the delivery tube into the acid mixture. Also rinse the outside of the delivery tube. Titrate the distillate mixture in the bottle with *N/70 sodium hydroxide* until the sodium alizarin sulphonate turns to a reddish color having a slightly purplish tinge. If one slips beyond the end point by accident, it is necessary to add exactly 5 cc of *N/70 acid* and to continue the titration, thus saving the estimation.

As suggested in the section on the use and calibration of apparatus, the end point may be more accurately determined by comparing it with a permanent standard.

It is almost impossible to procure commercial grades of sodium sulphate and sulphuric acid which do not contain small amounts of nitrogen so it is desirable to run controls occasionally without the added urine and to subtract the control figure from the calculated amount of standard acid combined with ammonia. It is also desirable to run controls occasionally on known nitrogenous solutions as a check on the technic.

Calculation Deduct the cc of alkali used in titration from the cc of acid taken (25), then deduct the control for ammonia in the reagents. This gives the cc of *N/70 NH₃* that has distilled over. Multiplying this by 0.001 (i.e., 5×0.0002) and by the urine volume (100 to get per cent) gives the grams of nitrogen in the 24 hour sample of urine.

Note Before doing the next estimation, dry the beads and the lower part of the Pyrex tube over a flame (air adsorbs to the glass and prevents bumping—Folin), but let the tube cool before adding the reagents to it

(3) *Reagents*—Sixty per cent sodium hydroxide Dissolve 100 grams of the pure sticks in 125 cc of distilled water and allow to settle Decant the clear liquid

Sodium sulphate sulphuric acid reagent Add an equal volume of sulphuric acid to water slowly while stirring and saturate with sodium sulphate If 1 cc of seleniumoxychloride (West and Brandon) is added per 500 cc, the reagent is even better and there will be no necessity for adding hydrogen peroxide

(b) *Colorimetric Method*—(1) *Technic*—If Nessler's solution is to be used, the procedure is the same as for the titration method up to the point of adding distilled water In this case, cool, add 25 cc of distilled water containing 1 cc of gum ghatti and, at once, 15 cc of Nessler's reagent and dilute to 50 cc The pyrex tube should have a mark on it corresponding to 50 cc While this is cooling add 1 cc of dilute standard ammonium sulphate solution to a similar test tube, graduated at 50 cc Add 1 cc of sodium sulphate sulphuric acid solution and 25 cc of distilled water containing 1 cc of gum ghatti and quickly to avoid separation of selenium 15 cc. of Nessler's reagent (measured with a graduate, not a pipette) Dilute to 50 cc and compare in a colorimeter

(2) *Calculation*—
$$\frac{\text{Mm of standard}}{\text{Mm of unknown}} \times 0.2 = \text{mg of nitrogen in the quantity}$$
 of urine (or blood filtrate) used Multiply this by 5 and by the urine volume (100 to get the percentage) and divide by 1000 to get the grams of nitrogen in the 24 hour urine

(3) *Reagents*—Gum ghatti To 5 gm of finely powdered gum ghatti (Eimer and Amend) in an Erlenmeyer flask add 250 cc of distilled water and shake at intervals for 2 to 4 hours Strain through a cloth and add 0.2 to 0.3 gm of benzoic acid dissolved in alcohol and shake at once

Nessler's solution (Folin) This is essentially a strongly alkaline solution of the double iodide of mercury and potassium Place 75 gm of potassium iodide and 55 gm of iodine in a 500 cc Florence flask Add 50 cc of water and about 75 gm of metallic mercury and (caution heat is generated) shake the flask vigorously until the red iodine solution has begun to become pale (10 to 15 minutes) Then cool in running water, continuing to shake until the red color is completely replaced by green Decant from the surplus mercury into a 1 liter flask and wash the mercury with repeated portions of distilled water, adding them to the solution in the flask Cool to room temperature and dilute to the mark From this stock solution of double iodide of mercury, make the Nessler's solution by adding 150 cc of it to 700 cc of 10 per cent carbonate free sodium hydroxide in a 1 liter flask and diluting to the mark with distilled water The 10 per cent sodium hydroxide may be made by dilution of 60 per cent It should be 2.5 times as strong as N/1 sulphuric acid when titrated with phenolphthalein as indicator

Standard ammonium sulphate solutions Dissolve 0.9428 gm of C P ammonium sulphate in distilled water in a 100 cc volumetric flask and dilute to the mark Ten cc of this diluted to 100 cc with distilled water gives a standard containing 1 mg of nitrogen in 5 cc

9 **Creatinine and Creatine**—I prefer the Folin colorimetric method with slight changes in the quantities used

(a) *Principle*—Creatinine has the property of reducing picric acid to picramic acid in quantitative amounts. Picramic acid in alkaline solution (sodium picramate) has a reddish-orange color which can be compared with standard N/2 potassium dichromate solution or pure creatinine standards. Creatine can be hydrolyzed to creatinine and the amount determined by difference.

(b) *Technic*—Creatinine. Measure exactly 2 cc of urine into a 100 cc volumetric flask, add 10 cc of saturated picric acid solution, 1 cc of clear 10 per cent sodium hydroxide and mix. After standing for 10 minutes fill to the mark with water and mix well. Compare some of this solution in a colorimeter with exactly N/2 potassium dichromate set at 8 mm as the standard. If the reading is less than 6 mm, make another estimation using 1 cc of urine but the same amount of reagents and multiply the calculated results by 2. If the reading is above 12 mm, use 4 cc of urine and divide the calculated results by 2. The temperature should be between 15° and 20° C and the estimation should be finished within 20 minutes after diluting.

Calculation Grams of creatinine in 24 hour specimen equals 8.1 divided by the average of four readings multiplied by the total cc of the 24 hour specimen divided by 1000. It has been determined that 2 mg of pure creatinine when treated as above and diluted to 100 cc yields a mixture 8.1 mm of which possesses the same colorimetric value as 8 mm of N/2 potassium dichromate solution. To convert creatinine to nitrogen of creatinine divide by 2.69.

Creatine To 2 cc of urine in a 100 cc volumetric flask add 2 cc of N/1 hydrochloric acid and place the flask in an autoclave at 115° to 120° for 30 minutes. Cool, add 2 cc of N/1 sodium hydroxide and proceed as in the creatinine estimation. The result obtained is creatinine plus creatine figured as creatinine. To get the creatine value subtract the creatinine value from the creatinine plus creatine.

(c) *Reagents*—(1) Saturated picric acid solution. Dissolve 2 grams of pure picric acid in 200 cc of distilled water by allowing it to stand at room temperature for two days shaking occasionally. *do not heat* because it becomes dark and may give a color 30 per cent darker than it should.

(2) N/2 potassium dichromate solution. Dissolve 2.455 grams of pure potassium dichromate in distilled water in a 100 cc volumetric flask and dilute to the mark. Keep it in a brown bottle.

10 **Chlorides (Volhard)**—(a) *Principle*—The urine is acidified with nitric acid and the chlorides are precipitated with a measured amount of standard silver nitrate solution (added in excess). The silver chloride formed is filtered off and the excess of silver nitrate in the filtrate is titrated back with standard thiocyanate (sodium potassium or ammonium) solution. Ferric alum is used as an indicator. A red color due to the formation of ferric thiocyanate indicates that an excess of thiocyanate is present and that the end point has been reached.

(b) *Technic*—Measure about 20 cc of distilled water and 2 cc of C P chloride free nitric acid into a 50 cc volumetric flask and add exactly 5 cc of urine and 10 cc of standard silver nitrate solution. Fill to the mark with distilled water and mix. Filter through a dry filter into a dry flask. Measure exactly 25 cc of the filtrate with a pipette into a titration flask, add 5 cc of 20 per cent ferric alum solution and titrate with standard sulphocyanate solution to a slight red color that remains after thorough mixing.

LABORATORY DIAGNOSIS

Calculation 10 cc of silver nitrate solution used) minus the cc of sulphocyanate used for titration (equals the cc of silver nitrate combined with chloride) multiplied by the factor (1 cc of silver nitrate equals 5 mg of chlorine or 8.23 mg of sodium chloride) gives the milligrams of chlorine or sodium chloride in the 5 cc of urine. Calculate the amount in the total urine.

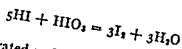
Note The sulphocyanate is only one half as strong as the silver nitrate which compensates for taking only one half of the filtrate for titration.

(c) *Reagents*—(1) Standard silver nitrate solution Dissolve 11.97 grams of C.P. silver nitrate in distilled water in a 500 cc volumetric flask, fill to the mark and mix. Keep it in a brown bottle.

(2) Standard sulphocyanate solution Dissolve 6 grams of ammonium (6.5 grams of sodium or 7.6 grams of potassium) sulphocyanate in about 800 cc of distilled water. With this solution titrate a mixture of 50 cc of distilled water, 5 cc of C.P. nitric acid, 10 cc of standard silver nitrate solution and 2 cc of ferric alum solution to a slight red color. Calculate the dilution necessary so that 20 cc of sulphocyanate will titrate 10 cc of silver nitrate solution e.g. if the titration is 18 cc, then 2 cc of water must be added to every 18 cc of the solution. After dilution titrate again.

11 Sugars—(a) *Shaffer Hartman Method as Modified by Haskins¹ and Holbrook* This method is recommended.

(1) Principle The reagent contains cupric sulphate in alkaline solution; this is reduced to cuprous oxide in amounts proportional to the amount of dextrose or other reducing substances present. Sulphuric acid in excess added after the heating process dissolves the oxide giving cuprous sulphate and liberates iodine from the potassium iodate by changing iodide and iodate to the corresponding acids which interact according to the equation



The amount of iodine liberated is determined by the amount of potassium iodate present since potassium iodide is present in excess. Iodine reacts with the cuprous sulphate changing it quantitatively to cupric sulphate. The amount of iodine which goes into combination in this reaction, therefore, bears a quantitative relation to the amount of reducing substances present. The free iodine left in solution after this reaction is titrated with the dilute standard sodium thiosulphate and the percentage of sugar corresponding to this titration is read directly from the table. The use of the table is made possible by this modification of the original Shaffer Hartman method. Different samples of the copper reagent gave control titrations with the exact N/200 sodium thiosulphate varying between 19.3 and 19.7 cc, which makes it impossible to use one table or curve for all of them. By this modification the thiosulphate is adjusted in each case to give a control titration of 19.5 cc, so that one table may be used for all estimations regardless of the value of the copper

¹ Haskins H. D. A New Table for Lactose (Milk or Urine) and Glucose (Blood or Urine) Calculation with Notes on Their Estimation. *Am J Med Sci* 172: 256-261 (Aug) 1926.
Haskins H. D. and Holbrook W. P. A Uniform Method for the Estimation of Glucose in Blood and Urine. *Northwest Medicine* 23: 355-357 (Aug) 1924.

reagent in terms of exact $N/200$ thiosulphate. This method gives results with a maximum error of 3 per cent if the directions are followed exactly.

(2) **Technic** Measure exactly 1 cc of urine into a 50 cc volumetric flask, or 2 cc into a 100 cc flask, fill to the mark with water and mix well. If the Benedict's qualitative test is less than 3 plus, measure exactly 5 cc of the diluted urine for estimation, if 3 plus, measure exactly 2 cc and add 3 cc of water, or if 4 plus, measure exactly 1 cc and add 4 cc of water, into an 8 by 1 inch test tube. Add exactly 5 cc of the micro copper reagent and mix well. Plug the tube loosely with non absorbent cotton or a glass bulb and place it, supported *vertically*, in a boiling water bath. After exactly 15 minutes heating, remove from the bath and cool rapidly, *without shaking*, to 30 to 40° C. Add 5 cc of $N/1$ sulphuric acid, mix and cork the tube loosely. This temperature is important for the effective reaction of the liberated iodine, especially in the case of the more concentrated sugar solutions. After 2 minutes titrate the excess of liberated iodine with the standard sodium thiosulphate solution. Add the standard solution somewhat rapidly until the liquid becomes a pale straw color, then add 0.5 cc of 2 per cent starch solution and titrate cautiously to the sudden disappearance of the starch iodine blue leaving a pale copper blue color. If in doubt as to the end point, note the reading of the burette and add another small drop. If no further change occurs, the end point had been reached. The color can be best judged by looking down through the mouth of the tube toward a white surface. When near the end point shake the tube¹ after the addition of each drop or insert a tight fitting cork and invert the tube.

Calculation In Table 18, find the per cent of sugar opposite the cc of thiosulphate used in the column U 5, U 2, or U 1 according to whether 5, 2 or 1 cc of dilute urine was used. In the case of lactose use column L 5 or L 2 (5 or 2 cc used) and for levulose or galactose multiply the dextrose value corresponding to the titration by the factor given at the end of the table. The table given here is slightly different from that originally published, being revised for low concentrations of dextrose, corresponding to titrations of over 17 cc.

Influence of Urinary Constituents—The slight reducing power of normal substances may be allowed for by deducting 0.1 for dextrose or 0.15 for lactose from the per cent figure. The presence of a moderate amount of protein causes no error.

¹ If many estimations are done in a day a convenient stirring device may be made from a second hand windshield wiper run by a suction pump and connected by a wire to a glass rod with a loop formed at right angles at one end. (West)

TABLE 18—PER CENT SUGAR CORRESPONDING TO CUBIC CENTIMETERS THIOSULPHATE USED FOR TITRATION¹

Cc	L ₂	L ₅	U ₁	U ₂	U ₅	B
19 2	0 50	0 20				
19 0	0 63	0 25	0 75	0 38	0 15	0 030
18 8	0 75	0 30	0 95	0 47	0 19	0 038
18 6	0 85	0 34	1 08	0 54	0 22	0 043
18 4	0 97	0 39	1 20	0 60	0 24	0 048
18 2	1 08	0 43	1 35	0 67	0 27	0 054
18 0	1 20	0 48	1 50	0 75	0 30	0 060
17 8	1 31	0 52	1 63	0 81	0 32	0 065
17 6	1 42	0 57	1 75	0 87	0 35	0 070
17 4	1 53	0 61	1 85	0 92	0 37	0 074
17 2	1 65	0 66	1 95	0 97	0 39	0 078
17 0	1 75	0 70	2 04	1 02	0 41	0 082
16 8	1 87	0 75	2 18	1 09	0 44	0 087
16 6	2 00	0 80	2 32	1 16	0 46	0 093
16 4	2 11	0 84	2 46	1 23	0 49	0 099
16 2	2 23	0 89	2 60	1 30	0 52	0 104
16 0	2 35	0 94	2 73	1 36	0 55	0 109
15 8	2 47	0 99	2 85	1 43	0 57	0 114
15 6	2 57	1 03	2 98	1 49	0 60	0 119
15 4	2 68	1 07	3 10	1 55	0 62	0 124
15 2	2 78	1 11	3 22	1 61	0 65	0 129
15 0	2 88	1 15	3 36	1 68	0 67	0 134
14 8	2 98	1 19	3 49	1 75	0 70	0 140
14 6	3 10	1 24	3 61	1 82	0 73	0 146
14 4	3 20	1 28	3 78	1 89	0 75	0 151
14 2	3 32	1 33	3 92	1 96	0 78	0 157
14 0	3 45	1 38	4 05	2 02	0 81	0 162
13 8	3 58	1 43	4 17	2 09	0 83	0 167
13 6	3 70	1 48	4 30	2 15	0 86	0 172
13 4	3 80	1 52	4 43	2 21	0 89	0 177
13 2	3 92	1 57	4 56	2 28	0 91	0 182
13 0	4 05	1 62	4 70	2 35	0 94	0 188
12 8	4 15	1 66	4 84	2 42	0 97	0 194
12 6	4 25	1 70	4 99	2 49	1 00	0 200
12 4	4 35	1 74	5 12	2 56	1 02	0 205
12 2	4 47	1 79	5 25	2 63	1 05	0 210
12 0	4 57	1 83	5 37	2 69	1 07	0 215
11 8	4 67	1 87	5 51	2 76	1 10	0 220
11 6	4 78	1 91	5 65	2 83	1 13	0 226
11 4	4 89	1 96	5 80	2 90	1 16	0 232
11 2	5 00	2 00	5 93	2 96	1 19	0 237
11 0	5 10	2 04	6 05	3 03	1 21	0 242
10 8	5 20	2 08	6 18	3 09	1 24	0 247
10 6	5 30	2 12	6 31	3 16	1 26	0 252
10 4	5 40	2 16	6 44	3 22	1 29	0 257
10 2	5 50	2 20	6 57	3 28	1 31	0 263
10 0	5 60	2 24	6 70	3 35	1 34	0 268

TABLE 18—PER CENT SUGAR CORRESPONDING TO CUBIC CENTIMETERS THIOSULPHATE USED FOR TITRATION—(Continued)

Cc	L ₂	L ₅	U ₁	U ₂	U ₅	B
9 8	5 70	2 28	6 85	3 42	1 37	0 274
9 6	5 80	2 32	6 98	3 49	1 40	0 279
9 4	5 90	2 36	7 12	3 56	1 42	0 285
9 2	6 00	2 40	7 24	3 62	1 44	0 290
9 0	6 10	2 44	7 35	3 67	1 47	0 294
8 8	6 20	2 48	7 46	3 73	1 49	0 298
8 6	6 30	2 52	7 57	3 79	1 51	0 303
8 4	6 40	2 56	7 69	3 84	1 54	0 307
8 2	6 50	2 60	7 79	3 89	1 56	0 312
8 0	6 62	2 65	7 90	3 95	1 58	0 316
7 8	6 73	2 69	8 01	4 00	1 60	0 320
7 6	6 85	2 74	8 12	4 06	1 62	0 325
7 4	6 95	2 78	8 23	4 12	1 65	0 330
7 2	7 07	2 83	8 38	4 19	1 67	0 335
7 0	7 20	2 88	8 50	4 25	1 70	0 340
6 8	7 30	2 92	8 64	4 32	1 73	0 346
6 6	7 42	2 97	8 78	4 39	1 76	0 351
6 4	7 55	3 02	8 92	4 46	1 78	0 357
6 2	7 65	3 06	9 07	4 53	1 81	0 363
6 0	7 75	3 10	9 21	4 61	1 84	0 368
5 8	7 87	3 15	9 36	4 68	1 87	0 374
5 6	8 00	3 20	9 50	4 75	1 90	0 380
5 4	8 10	3 24	9 64	4 82	1 93	0 386
5 2	8 20	3 28	9 78	4 89	1 96	0 391
5 0	8 32	3 33	9 92	4 96	1 98	0 397
4 8	8 43	3 37	10 06	5 03	2 01	0 403
4 6	8 55	3 42	10 20	5 10	2 04	0 408
4 4	8 65	3 46	10 34	5 17	2 07	0 413
4 2	8 75	3 50	10 48	5 24	2 10	0 419
4 0	8 85	3 54	10 61	5 31	2 12	0 424
3 8	8 95	3 58	10 75	5 38	2 15	0 430

L₂—Per cent anhydrous lactose if 2 cc is used for estimationL₅—Per cent anhydrous lactose if 5 cc is used for estimationU₁—Per cent anhydrous dextrose if 1 cc of diluted urine is usedU₂—Per cent anhydrous dextrose if 2 cc of diluted urine is usedU₅—Per cent anhydrous dextrose if 5 cc of diluted urine is used

B—Per cent anhydrous dextrose in blood

Multiply dextrose values by 1 06 for levulose and by 1 37 for galactose

* Extracted by permission from the Am J Med Sci 172 256 (August) 1926

(3) Reagents Microcopper reagent Dissolve each chemical separately (a) 40 gm of C P anhydrous or 47 gm of pure monohydrated sodium carbonate in 400 cc of warm distilled water (b) 5 gm of clear crystals of C P copper sulphate in 100 cc of water (c) 7 5 gm of pure tartaric acid in 100 cc of water (d) 0 700 gm of pure potassium iodate (weighed accurately) in 100 cc of water (e) 10 gm of potassium iodide in 100 cc of water (f) 18 4 gm of pure neutral potassium oxalate in 100 cc of water

When all are dissolved, mix (c) with (b) and pour the mixture slowly with stirring into (a). Combine (d), (e), and (f) and pour at once into the carbonate copper mixture. Transfer to a 1 liter volumetric flask. Rinse all the dissolving beakers with small portions of water and add to the solution in the flask. When cooled, fill to the mark and mix. On standing, a little sediment will be deposited, use the clear top liquid for estimations. If the chemicals are pure, the reagent run as a control will give the same titration whether heated in a bath 15 minutes or not heated. New microcopper reagents¹ have been proposed, I have not found that they have any advantage for clinical purposes. They can not be used with the table for calculation.

Standard thiosulphate. Dissolve 26 gm of sodium thiosulphate crystals and 0.5 grams sodium carbonate in 1000 cc of water which makes a solution stronger than N/10. This strong stock solution weakens very slowly after the first 2 days. Keep it in a brown bottle in a cool place. Make a fresh dilution of this for use in titration each day. Determine the ratio of dilution once in 2 weeks, as follows. Measure 5 cc of the strong stock solution into a 100 cc volumetric flask, add water to the mark, and mix. With this solution titrate 5 cc copper reagent plus 5 cc of water after adding acid as in an estimation. When titrations agree within 0.1 cc, prepare another dilution such that 19.5 cc will be required for titration of the control. For example, if the titration was 19.0 cc, dilute 5 cc to 102.6 cc, i.e., $(19.5 \text{ divided by } 19.0) \times 100$. Table 18 can be used only when the final control titration is 19.5 cc or the titration is corrected to this value. The dilute thiosulphate (about N/200) will remain unchanged for a considerable time if there is added to it 20 mg of anhydrous sodium carbonate per 100 cc of solution. The carbonate may be added as solution before filling to final volume. 0.5 cc. of 4 per cent solution for 100 cc of thiosulphate, or 2 cc of 10 per cent solution for 1 liter.

Starch solution. Mix 2 gm of soluble starch with 10 cc of water and pour it into 90 cc of boiling water, mix and boil 1 minute. Add a few drops of toluol as a preservative.

(b) *Benedict's Method*—This method is not nearly as accurate or convenient as

more than a 1 plus reduction, indicating the presence of over 0.5 per cent sugar with Benedict's qualitative test make an exact dilution so that the concentration is between 0.25 and 0.5 per cent sugar. For instance if a trial titration gives the result 7 cc, dilute the urine with an equal volume of water (dilution factor = 2) and titrate again.

Into a 200 cc Pyrex Erlenmeyer flask measure with a pipette or automatic burette exactly 25 cc of the reagent add a pinch of *pounded pumice* and about 4 grams of *monohydrated sodium carbonate*. Insert a two hole rubber stopper which has in one hole a bent tube to convey away the steam during the boiling. Place the flask on wire gauze supported by a tripod and insert the tip of the burette, filled with the urine, into the other hole of the stopper. A large asbestos pad, about 10 inches in diameter and with a 2 inch hole in the center, placed under the flask will protect the hands from the flame during the titration. Bring the reagent to boiling and keep it continuously and vigorously boiling throughout the estimation. No shaking of the mixture is necessary. As soon as the reagent boils begin adding the urine and add it continuously as fast as drops can be counted but never in a stream. When a chalky white precipitate appears in the blue mixture add the urine drop by drop having a slight interval between the drops and finally when the mixture is of a very pale greenish color have a distinct pause between drops. The end point is a yellowish white turbid liquid with no tinge of green.

Calculation 25 cc of Benedict's reagent is reduced by 0.05 gm of dextrose or 0.067 gm of lactose, therefore $\frac{0.050}{Z} \times 100 \times \text{dilution factor} = \text{per cent of dextrose in the original sample}$ where Z is the number of cc of urine required to reduce the 25 cc of reagent.

This method will give results within 10 per cent of the actual percentage of sugar present if the directions as given are closely adhered to and frequent checks on the technic are made by titration of sugar solutions of known concentration. However, as ordinarily done, errors of 30 per cent are not uncommon.

(3) Reagent Benedict's reagent. Dissolve exactly 18 gm of noneffloresced copper sulphate crystals in 100 cc of distilled water. Dissolve 100 gm of monohydrated or 200 gm of crystals of sodium carbonate in about 575 cc of water with the aid of heat add 200 gm of sodium citrate and 105 gm of sodium or 125 gm of potassium thiocyanate and stir or shake until dissolved. Filter if the solution is not clear use distilled water to take the salts out of the filter paper. Bring the volume to about 800 cc. Pour the copper sulphate solution into it with constant shaking or stirring use 25 cc of water to rinse the rest of the copper solution out of the flask. Transfer to a 1 liter volumetric flask add 5 cc of 5 per cent potassium ferrocyanide solution rinse all the reagent from the previous container into the flask with water and when cooled to room temperature fill to the mark with water.

(c) *Polariscopic Determination*—This method gives results sufficiently accurate for most clinical purposes but requires the use of an expensive apparatus. Erroneous results may be obtained due to the presence of optically active substances other than sugar such as glycuronates. Before doing a polariscopic examination the urine must be thoroughly decolorized by vigorous shaking with some adsorbent substance such as infusorial earth and filtration or better by the following technic. Mix exactly 10 cc. of urine with acetone until the volume becomes exactly 12.5 cc add 1 gram of Merck's blood charcoal and shake continuously for 5 minutes then filter and use the filtrate for the determination. If the latter method is used correct

for the dilution by the acetone by multiplying the calculated result by 1.25. The formula used for calculating is

$$\frac{\text{Observed rotation} \times 100}{\text{Specific rotation} \times \text{tube length in decimeters}} = \text{per cent of sugar}$$

The specific rotation of dextrose is $+52.5^\circ$, of lactose is $+56^\circ$, and of galactose is $+81.5^\circ$

12 Galactose Tolerance Test—(a) *Principle*—In diffuse liver disease less galactose is converted to glycogen and more is excreted

(b) *Technic*—The patient is instructed to come to the laboratory without breakfast. On arrival, have the patient void and test the urine for reduction. If negative, give 40 gm of galactose in 10 per cent solution and collect all the urine voided for 5 hours or until the reduction test is again negative. Mix all samples showing reduction, measure the volume, and make a quantitative sugar estimation as directed (p. 372). Multiply the factor for dextrose in Table 18 by 1.37 to get the per cent of galactose. Calculate and report the total grams excreted. Normally this should not exceed 3 gm.

13 Alkali Tolerance Test—This test was first used by Sellards.¹ It is reliable for showing the absence of acidosis. Generally it detects an existing acidosis and gives a rough idea of the degree of acidosis, provided there is no impairment of kidney function. It is now rarely used.

(a) *Principle*—Sodium bicarbonate is administered in small amounts by mouth until the reaction of the urine changes from acid to alkaline or, as I prefer, until there is a rise of 0.7 to 1.0 in the pH figure. The amount of bicarbonate is then noted.

(b) *Technic*—Have the patient empty the bladder and determine the approximate pH with Squibb's nitrazine paper. If it is alkaline, acidosis is not present. If it is acid, give 5 grams of sodium bicarbonate dissolved in water every half hour until the pH changes. Have the patient void every 15 minutes and test the urine with nitrazine paper. Record the total grams of sodium bicarbonate required to produce an increase of about 1 in pH.

Normals require 10 grams or less, so that if this amount does not produce a significant change in the reaction of the urine, the test should be stopped and an alkali reserve estimation be made on the patient's blood.

¹ Sellards, A. W. The Determination of the Equilibrium in the Human Body between Acids and Bases with Especial Reference to Acidosis and Nephropathies. Bull. Johns Hopkins Hosp. 23: 289-302 (Oct.) 1912.

14 Total Titratable Acidity (Folin) —(a) Principle—The urine is titrated with standard sodium hydroxide solution in the presence of potassium oxalate using phenolphthalein as an indicator. The potassium oxalate is added to precipitate the calcium which would otherwise interfere with the end point, due to the precipitation of calcium phosphate on neutralization of the urine. Acid phosphate is changed to alkaline (disodium) phosphate. The end point is distinctly on the alkaline side of the neutral point.

(b) Technic—In a beaker or titration flask, mix 2 cc of saturated (30 per cent) neutral potassium oxalate solution with 25 cc of distilled water and 0.5 cc of 1 per cent alcoholic phenolphthalein solution. Add to this mixture dilute sodium hydroxide (e.g., N/70) until a faint pink is secured. Then add exactly 10 cc of the urine and titrate with N/20 sodium hydroxide to a slight but easily distinguished pink color that remains at least 15 seconds after thorough mixing.

Calculation Divide the cc of alkali used by 2 to give the cc of N/10 sodium hydroxide. Calculate the total titration value of the 24 hour sample.

Note If a number of urines are to be titrated it is advisable to use a standard color mixture for comparison so as to secure the same end point in all titrations. This may be prepared by adding a red dye such as amaranth to a mixture of urine oxalate and water.

15 Quantitative Determination of Sulfanilamide or Sulfapyridine in Urine—Make an accurate 1-10 dilution of the urine with distilled water. Measure 10 cc of this diluted urine into a flask and proceed as described under determination of sulfanilamide or sulfapyridine in blood (page 417). Multiply the calculated results by 10.

16 Quantitative Determination of Urobilinogen in Urine (Sparkman¹) —(a) Principle—The urobilinogen of freshly voided urine reacts with Ehrlich's aldehyde solution to form a colored compound and the intensity of the color is compared with that of an artificial standard of known urobilinogen equivalent.

(b) Technic—If the 24 hour excretion is desired, collect the urine as advocated by Watson in a brown bottle containing 100 cc of petroleum ether and 5 gm of anhydrous sodium carbonate. A freshly voided specimen may be used at once for the determination without addition of a preservative. To 25 cc of urine in a flask add 1 gm of anhydrous calcium chloride, shake and filter. The calcium chloride precipitates and removes bile pigment. A drop of fuming nitric acid added to the precipitate on the filter will give the characteristic colored rings if bile pigment is present and is an even more sensitive test for

¹ Sparkman R. Studies of Urobilinogen. I. A Simple and Rapid Method for the Quantitative Determination of Urobilinogen in Stool and Urine. II. Normal Values for Urobilinogen Excretion in Single Specimens of Urine and Stool. III. The Clinical Value of Determinations of the Urobilinogen Content of Single Specimens of Urine and Stool. Arch Int Med 63: 858-883 (May) 1939. Reproduced by permission of the author and publisher.

bile pigment than the Gmelin-Rosenbach method To 10 cc of the filtrate in a test tube add 1 cc of the aldehyde reagent and mix thoroughly by inversion At the end of 5 minutes, match in a colorimeter against the artificial standard nearest the unknown in color

(c) *Calculation* — $\frac{\text{Reading of the standard}}{\text{Reading of the unknown}} \times \text{the factor} = \text{the mg of urobilinogen per 100 cc of urine}$ The factor is 8.2 with the strong standard, 2.4 with the intermediate standard, and 0.9 with the weak standard

(d) *Reagents* — Ehrlich's aldehyde reagent — To 10 grams of paradimethyl aminobenzaldehyde, add a mixture of 75 cc of distilled water and 75 cc of C P hydrochloric acid This keeps well

Artificial standards These keep well

Strong standard Prepare an accurate 4 per cent solution of gold chloride and 10 per cent solution of sodium bromide To 10 cc of 4 per cent gold chloride add 10 cc. of 10 per cent sodium bromide and dilute to 150 cc

Intermediate standard Transfer 50 cc of strong standard to a 100 cc volumetric flask and dilute to the mark with distilled water

Weak standard Dilute 50 cc of the intermediate standard to 100 cc with distilled water

17 **Table of Normal Findings** — As pointed out in the introduction to Chapter II, it is impossible to define a normal urine unless the variable factors of diet, fluid intake, plasma composition, etc are known In Table 19 the usual findings in the urine of the average, healthy adult under the average living conditions in the United States are given

TABLE 19 — NORMAL URINE VALUES

Volume	800 to 1800 cc per 24 hours
Specific gravity	1.010 to 1.025 on 24 hour sample
Total solids	30 to 60 grams per 24 hours
Total nitrogen	10 to 16 grams per 24 hours
Urea nitrogen	7 to 13 grams per 24 hours or 75 to 90 per cent of the total nitrogen
Ammonia nitrogen	0.3 to 1.0 gram per 24 hours or less than 10 per cent of the total nitrogen
Creatinine	1.0 to 1.8 grams per 24 hours
Uric acid	0.5 to 0.7 gram per 24 hours
Sodium chloride	10.0 to 16.0 grams per 24 hours
Total titratable acidity equivalent to pH	150 to 400 cc of N/10 acid per 24 hours 5.5 to 7.0
Phenolsulphonphthalein excretion	First specimen 40 to 60 per cent Second specimen 20 to 25 per cent Total for 2 specimens 60 to 80 per cent
Galactose tolerance	Less than 3.0 gm excreted

SECTION IV BLOOD CHEMISTRY

A Collection and Labelling of Specimens—All blood for quantitative chemical analysis must be collected after a fasting period, preferably in the morning before breakfast, unless otherwise specified. The blood should be withdrawn from the vein as soon as possible after the tourniquet is applied because venous stasis tends to produce a rapid change in the composition of the blood. Dry powdered potassium oxalate,¹ 2 mg per 1 cc of blood, is recommended as an anti-coagulant in those cases where whole blood or blood plasma is used for the determination. Mix the blood rapidly and thoroughly with the oxalate by holding the corked test tube horizontally and tapping it with one hand and place it in the icebox unless the determination is begun immediately. Estimations of urea, creatinine, and sugar may be inaccurate if the blood stands at room temperature for 4 hours or at 8° C for 24 hours. Addition of 10 mg of monochlorobenzene² and 10 mg of sodium fluoride per cc of oxalated blood will prevent these changes for 6 days or more and will permit the mailing of specimens. If a protein free filtrate is to be used, it should be prepared immediately, then if it is saturated with toluol and placed in the icebox, it will keep well for at least 24 hours. All specimens should be labelled with the patient's name in full, the minute, hour and day and the tests desired.

B Blood Urea Nitrogen (Van Slyke and Cullen modification of Marshall's urease method)—The urease method on the whole blood using the same aeration apparatus as for urine urea is recommended.

1 **Principle**—This is identical with that of the urine urea determination.

2 **Technic**—First make sure that the blood tube (No. 2) is free of alkali as directed in the urine urea determination on page 365. When the tube is neutral, measure into it exactly 4 cc³ of well mixed whole blood, and add 1.5 cc of 2.5 per cent *acid potassium phosphate* solution as a buffer, 4 drops of *caprylic alcohol*, and 1 cc of 10 per cent *urease solution* or rub up 0.1 gram or 1 tablet of urease with 1 cc of water, pour into the tube and rinse the mortar with the buffer solution. The urease glycerin solution described under the urine urea nitrogen may be used instead. Connect the tube with the apparatus and

¹ See p. 461 for a rapid method of preparing a large number of tubes.

² Lewis R. C. and Mills G. E. The Comparative Value of Monochlorobenzene and Thymol When Used with Fluoride as Preservatives of Blood for Chemical Analysis. *Am J Clin Path* 3: 17-28 (Jan) 1933.

³ If a very high estimation is expected use 2 cc and multiply the result by 2.

immerse the lower portion in a water bath at 50 to 55° C. Allow thirty minutes for enzyme action. Into the acid tube (No. 4) measure exactly 25 cc of *N/70 sulphuric acid*, add 4 drops of caprylic alcohol and one drop of 1 per cent sodium alizarin sulphonate solution and connect with the apparatus.

After thirty minutes, run the air stream slowly for one-half minute and stop, disconnect the blood tube but do not remove it from the apparatus, add 6 cc of *potassium carbonate oxalate reagent* as for urine urea and connect with the apparatus again. Run the air stream slowly for 5 minutes and rapidly for 30 minutes. Then transfer the acid solution, with proper rinsing, to a titration flask and titrate it with *N/70 sodium hydroxide*.

Calculation Subtract the cc of sodium hydroxide used for the titration from the cc of acid taken, then deduct the control for ammonia in the reagents. Now multiply this figure by 5¹. The result is mg of urea nitrogen per 100 cc of blood. There is so little ammonia in the blood that no correction is made for it.

Note If only blood filtrate is available, 10 cc of filtrate which equals 1 cc of blood may be used instead of the 4 cc of blood, using *N/280 acid* and alkali which equals 1:1 to 4 dilution of *N/70*. This is simpler and more dependable than the use of the distillation urea methods.

If Nesslerization methods are preferred, incubate 10 cc of filtrate with urease and buffer, then add gum ghatti and 15 cc of Nessler's reagent and dilute to 50 cc as directed on page 370 and proceed as directed for the colorimetric technic of total nitrogen estimation in urine. Calculate as directed there but deduct a control determined on the reagents alone.

C The Blood Urea Clearance²—This is a more laborious and expensive test than the blood urea nitrogen, but it is more sensitive to slight grades of impaired kidney function.

1 Principle—Under certain conditions the rate of urea excretion by the kidney is directly proportional to the level of the blood urea nitrogen. In other words, the amount of urea nitrogen excreted in one minute corresponds to the urea nitrogen content of a constant volume of blood. This constant volume of blood, which might be cleared of urea in one minute, is called the urea clearance (C). When the urine volume is large, the rate of urea excretion is maximum and is unin-

¹ One cubic centimeter of *N/70 ammonia* contains 0.2 mg of nitrogen. 4 cc or 1/25 of 100 cc of blood are taken for estimation, 25 multiplied by 0.2 equals 5.0 therefore each one cc of *N/70 acid* used is equivalent to 5 mg of nitrogen per 100 cc of blood.

² Moller E. McIntosh J. F. and Van Slyke D. D. Studies of Urea Excretion. II Relationship Between Urine Volume and Rate of Urea Excretion by Normal Adults. *J Clin Invest* 6: 427-465, 1929.

Peters J. P. and Van Slyke D. D. Quantitative Clinical Chemistry. Pp 335-369. Vol I. The Williams & Wilkins Co. Baltimore 1932.

fluenced by variations in volume of urine formation per minute and the maximum urea clearance (C_m) is calculated. When the rate of urine formation falls below a certain limit, called the "augmentation limit," the rate of urea excretion also falls and has been shown to vary in proportion to the square root of the urine volume per minute. The augmentation limit has been shown to average about 2 cc per minute. When the urine volume is below 2 cc per minute the urea clearance is calculated to that which would occur if the urine volume were 1 cc per minute. This is called the standard clearance (C_s). The urine volume of 1 cc per minute was chosen for convenience in calculation and because it is approximately the average normal rate of urine formation. It is evident that the data necessary for calculation of urea clearance are a blood urea nitrogen (B), a urine urea nitrogen (U), and the rate of urine formation (V).

Since the only other variables are the blood supply to the kidneys and the amount of functioning renal tissue, the urea clearance test gives a quantitative expression of renal function. The amount of kidney tissue normally present has been shown to be proportional to the surface area (SA), and, therefore, by comparing the surface area with the average normal, a corrected urea clearance is obtained which can be compared with that of the average normal adult and is expressed as per cent of the average normal.

2 **Technic**—The test may be done at any time, but it is best to do it in the morning before breakfast so that the blood urea nitrogen will be of clinical value. The patient may have fluids as desired aside from diuretics such as coffee or tea. The patient is instructed to empty the bladder completely, is catheterized if necessary, and the time is noted to the nearest minute. This urine is discarded. About 55 minutes later, 5 cc of blood is withdrawn from a vein, oxalated, and used for the blood urea nitrogen estimation. About an hour after the bladder was emptied, it is emptied completely a second time into a clean, dry receptacle and the time of complete emptying noted. Similarly a second urine fraction is collected at the end of another hour. These two urine samples are saved, corked, and labelled with the exact times of collection in minutes (e.g., first specimen 63 minutes, second specimen 58 minutes). The patient's height and weight are recorded.

In the laboratory, the urine volumes are accurately measured and a urea nitrogen estimation is done on each sample of urine and on the blood according to the technics previously given. Express the urea nitrogen in milligrams per 100 cc of urine. Determine the rate of urine formation per minute for each specimen by dividing the volume

in cubic centimeters by the time in minutes. It has been shown that the urea plus ammonia nitrogen in the urine is satisfactory for calculation of the urea clearance because the ammonia in urine is formed from urea in the kidney. This dispenses with the necessity of ammonia nitrogen estimations or extraction of the urine with permutit.

3 Calculation—Multiply the observed volume in cc per minute by $\frac{1.73}{SA}$ ¹ to get the corrected urine volume (V), where SA is the patient's surface area obtained from the table on page 426. If the corrected urine volume exceeds 2 cc, calculate the maximum urea clearance (Cm) by the formula $Cm = \frac{UV}{B}$, where U equals the urine urea nitrogen in milligrams per 100 cc, B equals the blood urea nitrogen in milligrams per 100 cc, and V equals the corrected urine volume per minute. If the corrected urine volume is less than 2 cc, calculate the standard urea clearance (Cs) by the formula $Cs = \frac{U}{B} \sqrt{V}$, where U, B, and V have the same significance as above. Divide the observed maximum clearance by 75 (the average normal Cm) and multiply by 100 to express the result in percentage of normal. To express the standard clearance as percentage of the normal, divide the observed clearance by 54 (the average normal Cs) and multiply by 100. If the urea clearances as calculated from the two specimens disagree by more than 30 per cent, an error is probable, and the test should be repeated. Report (1) the blood and urine urea nitrogens in milligrams per 100 cc, (2) the urine volume, time and cc per minute for each specimen, (3) the patient's height, weight and surface area, (4) the corrected urine volume per minute, and (5) Cm or Cs and the percentage of normal for each specimen and the average of the two.

EXAMPLE OF CALCULATION PATIENT IS 67 IN TALL AND WEIGHS 160 LB

Blood Urea Nitrogen—8.25 mg per 100 cc

First Specimen

Second Specimen

Volume—108 cc Time—63 min

Volume—130 cc Time—60 min

Urine urea nitrogen—328 mg per 100 cc

Urine urea nitrogen—267 mg per 100 cc

Observed volume = $\frac{108}{63} = 1.71$ cc per min

Observed volume = $\frac{130}{60} = 2.17$ cc per min

Corrected volume = $1.71 \times \frac{1.73}{1.80} = 1.65$ cc

Corrected volume = $2.17 \times \frac{1.73}{1.80} = 2.08$ cc

$Cs = \frac{U}{B} \sqrt{V} = \frac{328}{8.25} \sqrt{1.65} = 50.88$ cc

$Cm = \frac{UV}{B} = \frac{267 \times 2.08}{8.25} = 67.31$ cc

Per cent of normal = $\frac{50.88 \times 100}{54} =$

Per cent of normal = $\frac{67.31 \times 100}{75} =$

94.2 per cent

89.7 per cent

¹ The average normal surface area of the adult male

EXAMPLE OF REPORT	HEIGHT—67 IN	WEIGHT—160 LB	SURFACE AREA—1 80 SQ M
	Blood Urea Nitrogen—8 25 mg per 100 cc		
	First Specimen		Second Specimen
Urine urea nitrogen	328 mg per 100 cc	267 mg per 100 cc	
Volume	108 cc	130 cc	
Time	63 min	60 min	
Cc per minute	1 71 cc	2 17 cc	
Corrected volume	1 65 cc	2 08 cc	
Cs	50 88	Cm 67 31	
Per cent of normal	94 2 per cent	89 7 per cent	
Average per cent	91 9 per cent		

D Preparation of the Protein-free Filtrate —1 **Tungstate Precipitation (Folin)** —The normal values given in this book are for this technic unless otherwise stated

(a) *Principle* —The total proteins of the blood are removed by precipitation with tungstic acid formed by the interaction of sodium tungstate and sulphuric acid and filtration. The filtrate contains all of the constituents of the blood determined by this system of methods

(b) *Technic* —Measure all liquids accurately since the filtrate is to be an exact 10 per cent dilution of the blood. If only one method is to be carried out, 2 cc of blood may suffice, 7 cc will furnish filtrate for all the Folin methods

Blood cubic centimeters	Distilled water cubic centimeters	Sodium tungstate (10 per cent) cubic centimeters	Sulphuric acid ($\frac{2}{3}$ N) cubic centimeters
2	14	2	2
5	35	5	5
7	49	7	7

Mix the blood and water in a flask and let it stand until well laked. Add the tungstate and mix, and then add the acid *slowly* while shaking. Haden recommends using N/12 sulphuric acid instead of adding the water and $\frac{2}{3}$ normal sulphuric acid separately. If this method is used the volume of N/12 sulphuric acid should equal eight times the volume of blood or sodium tungstate used or the sum of the figures for distilled water and acid as given in the table. The acid is added before the tungstate in this technic. It saves one measurement but there is somewhat more danger of having to refilter to secure a clear filtrate. Cork the flask, shake it vigorously, and let it stand 10 or 15 minutes. The precipitate should become brownish red and almost no foam should form on shaking. If these conditions are not met, add a little 10 per cent sulphuric acid a drop at a time and shake vigorously.

The mixture should not become more than faintly acid to congo red paper

Filtrate may be prepared from plasma or serum¹ if treated as follows use one half quantity of tungstate and of $\frac{3}{4}$ normal sulphuric acid and increase the water to correspond, e.g., for 5 cc of plasma use 40 cc of water and 2.5 cc each of tungstate and acid. Remember that normal values for plasma and whole blood are different

Filter, using a small retentive filter paper. Whatman No. 2 filter paper is recommended. If the filtrate is not clear and colorless, pour it back once or twice onto the filter to see whether it will become clear. If unsuccessful, return the precipitate and filtrate to the flask, treat with more acid and filter.

(c) *Reagents* —(1) Two thirds normal sulphuric acid is prepared by diluting 100 cc of N/1 acid with 50 cc of distilled water. One twelfth normal sulphuric acid may be prepared by accurate dilution of N/1 acid.

(2) Sodium tungstate solution (10 per cent). C.P. sodium tungstate should be used and it should go into solution readily. The reagent must be tested for excess of carbonate as follows: titrate 5 cc with $\frac{2}{5}$ normal sulphuric acid using methyl orange, until the yellow color changes to a slightly reddish yellow, between 3 and 3.3 cc should be required. If the titration is greater, add twice normal acid made by diluting 11.5 cc of C.P. sulphuric acid to 200 cc, in sufficient amount to the whole stock of tungstate solution to bring the alkalinity down to the proper limits and titrate again. Also test the tungstate against blood and $\frac{3}{4}$ normal or N/12 sulphuric acid, no additional acid should be required to produce complete precipitation.

2. The Zinc Filtrate for True Sugar Estimation (Somogyi) —To 2 cc of oxalated venous blood in an Erlenmeyer flask add 16 cc of solution I, mix until clear, and add 2 cc of solution II. Shake thoroughly, allow to stand a few minutes and filter or centrifugate. Use the clear filtrate or supernatant liquid for the blood sugar estimation by any of the methods given. The values will be true sugar and will average about 21 mg lower than the sugar estimations on the tungstate filtrates the difference being due to non dextrose reducing substances which are not removed by the tungstate.

(a) *Reagents* —Solution I. Dissolve 12.5 gm of zinc sulphate ($\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$) in water. Add 31 cc of N/1 sulphuric acid and dilute to 1 liter.

Solution II. Dissolve 2.5 gm of sodium tungstate in 75 cc of N/1 sodium hydroxide and dilute to 100 cc. It should require 3.35 to 3.4 cc of solution II to neutralize 25 cc of solution I to phenolphthalein.

E. Determinations on Protein free Filtrates of Blood, Plasma, or Serum —1. **Non-protein Nitrogen of Blood** —Results of this test almost parallel at a higher level the blood urea nitrogen which

¹ I preparation of a filtrate from unclotted blood (Folin) is not recommended, as it gives neither plasma nor whole blood values but values for an unmeasured volume of plasma. The great variations in cell volume are not considered in this method.

yields the same information and is more easily determined. Another disadvantage is that the non protein nitrogen method determines several substances, all of which may vary individually, while the urea method determines a single substance.

(a) *Principle*—This is exactly the same as for the total nitrogen of urine (p 367)

(b) *Technic*—Proceed exactly as directed for total nitrogen in urine, except that 10 cc of blood filtrate is used instead of urine.

Calculation Subtract the cc of sodium hydroxide used for titration from the cc of acid taken, then deduct the control for nitrogen in reagents. Multiply this by 20 (i.e., $0.0002 \times 100 \times 1000$) if N/70 solutions were used. The result is mg of total non protein nitrogen per 100 cc of blood.

If the Nesslerization method is used, multiply the mg of nitrogen in the blood filtrate used by 100 to get the mg of nitrogen per 100 cc of blood since 10 cc of filtrate equals 1 cc of blood.

2 **Blood "Creatinine"**—This estimation is desirable whenever the blood urea nitrogen is found to be above 20 mg.

(a) *Principle*—This is the same as for creatinine of urine (q.v.) It is almost certain that the color change in the picrate reagent is not due to creatinine but this fact does not impair the clinical value of the test.

(b) *Technic*—Measure exactly 10 cc of protein free filtrate into an 8 by 1 inch test-tube. Into another tube, measure exactly 5 cc of standard creatinine solution, add 5 cc of N/10 sodium hydroxide and 10 cc of distilled water. Now add the picrate reagent to both tubes, 5 cc to the blood filtrate and 10 cc to the standard, and mix. After 8 minutes start the color comparison, setting the standard at 20 mm, and finish the readings within 15 minutes after adding the reagent.

Calculation
$$\frac{\text{Mm standard (i.e., 20)}}{\text{Mm unknown}} \times 15^* = \text{mg of creatinine in 100 cc of blood}$$

If the estimation is high, repeat as above but use 5 cc of filtrate plus 5 cc of water, or use a standard of double strength, 10 cc of creatinine solution plus 10 cc of N/10 sodium hydroxide plus picrate reagent, in either case use 3 instead of 15 in calculating.

(c) *Reagents*—(1) Picrate reagent. Make this fresh for each estimation. Mix 15 cc of saturated picric acid solution with 3 cc of clear 10 per cent sodium hydroxide.

* Five cubic centimeters of standard creatinine solution is equivalent to 0.03 mg of creatinine. The standard is one half as strong as the unknown (30 cc 15 cc) and filtrate equivalent to 1 cc of blood is taken for estimation. Therefore the derivation of the figure for calculation is 0.03 divided by 2 times 100 equals 15.

ide (exactly 25 times N/1) Use the best grade of picric acid and prepare the saturated solution as directed under urine creatinine

(2) *Standard creatinine* Estimate accurately the creatinine content (see p 371) of normal urine that has stood a week or more after having been acidified with about 4 cc CP hydrochloric acid for 100 cc The creatinine content is constant for a long time Calculate the amount of urine that contains 0.6 mg of creatinine Measure this amount accurately with a pipette marked for 0.01 cc into a 100 cc volumetric flask, add 50 cc of N/5 hydrochloric acid, dilute to the mark with distilled water, mix and add toluol Make this dilute standard every few months

It is preferable to use pure creatinine dissolve exactly 0.1 gm of pure creatinine in 80 cc of N/10 hydrochloric acid in a 100 cc volumetric flask and make up to the mark with distilled water For the dilute standard, dilute exactly 0.6 cc of this with N/10 hydrochloric acid to 100 cc in a volumetric flask 5 cc of the dilute standard contains 0.03 mg of creatinine

3 *Blood Uric Acid* (as modified by Haskins and Holbrook¹)—For this estimation it is quite important that the sodium tungstate be adjusted to correspond to the 2/3 normal acid so that the blood filtrate shall not be strongly acid, otherwise some uric acid may be precipitated with the protein It is advisable to test the tungstate and acid against oxalated blood Add a drop of methyl orange solution to a few cc of the protein free filtrate, there must be no tinge of red in the color indicating that the pH is 4.5 or more Loss of uric acid is thus avoided

Of all the numerous changes of Folin's uric acid method that have been proposed only two seem valuable (1) direct estimation as suggested by Benedict² (Folin³ has accepted this change), and (2) precipitation by zinc chloride as suggested by Morris⁴ The recommended method as given below is an adaptation of these three methods which has proved dependable

(a) *Direct Estimation Method* (Folin Benedict)—(1) *Principle* Uric acid reduces phosphotungstic acid solution quantitatively with the formation of a blue color which can be estimated colorimetrically The lithium sulphate tends to prevent turbidity The cyanide intensifies the blue color and thus makes the method more sensitive

(2) *Technic* Have a boiling bath ready Measure exactly 5 cc of blood filtrate and exactly 0.5 cc of modified standard uric acid solution into a test tube Into a second tube measure exactly 1 cc of modified standard uric acid solution and 4.5 cc of water Now add to each of the tubes 3 drops of 20 per cent lithium sulphate solution and 2 cc of sodium cyanide solution (Caution! Poison!) Add last directly to the liquid so that it does not flow down the wall, 1 cc of uric acid reagent Mix and after standing 2 minutes heat in the bath exactly 2 minutes Cool at once under the tap to room temperature dilute without delay to 25 cc and mix Estimate at once, setting the standard which is in the second tube at

¹ Haskins, H D, and Holbrook, W P. Blood Uric Acid. Comparative Results by Three Methods and Technic Necessary for Accurate Estimation. J Lab and Clin Med 11: 377-381 (Jan) 1926

² Benedict, S R. The Determination of Uric Acid in Blood. J Biol Chem 51: 187-207 (March) 1922

³ Folin, O. and Wu, H. A System of Blood Analysis. J Biol Chem 38: 81-110 (May) 1919

⁴ Morris, J L, and Macleod, A G. Colorimetric Determination of Uric Acid. J Biol Chem 50: 55-63 (Jan) 1922

20 mm The readings must come between 10 and 40 mm If below 10 mm repeat the estimation using 2.5 cc of filtrate and 2.5 cc of water, and multiply the calculated results by 2 Calculation $\left(\frac{20}{\text{Mm unknown}} \times 4 \right)$ minus 2 = mg of uric acid in 100 cc of blood

Note 1 If an estimation above normal is expected either run 3 tubes the third tube having 5 cc of blood filtrate without added uric acid but 0.5 cc of water instead or else run the 2 tubes as above except that water is added to the first tube instead of uric acid If uric acid is not added the 2 mg must not be deducted during the calculation

The addition of known uric acid to the unknown is a modification by Haskins and Holbrook which gives very good results if the estimation is not above 4 mg If the result is above normal do a check method

Note 2 The direct method is most generally used at present There is some evidence that in certain cases this estimation is too high because of the presence of substances other than uric acid that give a blue color with the reagents It is possible that Folin's new improved uric acid reagent¹ which is freed of molybdenum may give accurate direct estimations Until this has been determined it is safer to repeat the estimation using the zinc chloride check method when the result is above normal by the direct method

(3) Reagents (a) Folin's uric acid reagent Dissolve 50 gm of C P sodium tungstate in 350 cc of distilled water add 40 cc of 85 per cent phosphoric acid, and heat the flask having a reflux tube attached Boil for 2 hours, cool and dilute to 500 cc

Note Folin's new improved reagent is not easily prepared Those who are not chemists should not try to make the reagent but purchase it

(b) Folin's sodium cyanide solution (Caution! Poison!) Dissolve 15 grams of purest sodium cyanide in 100 cc of N/10 sodium hydroxide Impurities cause the fresh solution to give considerable blue color interfering with the estimation After about 2 weeks the solution gives little color When 2 or 3 months old it should be discarded since NH_4 is produced which makes it inefficient or else purified by aeration Very old solutions give an improper type of blue color The best plan is to make a small quantity of solution every month

(c) Folin's standard uric acid solutions (a) Stock Solution Dissolve 0.3 grams of lithium carbonate in 60 cc of hot distilled water filter off any undissolved impurity and add 30 cc of water and warm to 65°C Warm a 500 cc measuring flask with hot water pour into it exactly 0.5 gram of pure uric acid and then the hot lithium carbonate solution rinsing the weighing beaker and the funnel with the latter Shake until the uric acid is dissolved then cool under the tap and add 300 cc of water Add 5 cc of Merck's formaldehyde and mix Add a cooled dilution of 7.5 cc of C P sulphuric acid in 50 cc of water Dilute with water to the mark and mix well This stock solution keeps well (b) Modified standard (Haskins) mix exactly 2 cc of stock solution 50 cc of water 5 cc of 2/3 normal sulphuric acid and 0.5 cc of formalin in a 100 cc volumetric flask and fill to the mark This is 5 times as strong as Folin's dilute standard Make the solution each month

¹ Folin O and Trimble H A System of Blood Analysis Improvements in the Quality and Method of Preparing the Uric Acid Reagent J Biol Chem 60 473-479 (June) 1924

(b) *Zinc Chloride Check Method* (Morris, modified by Haskins and Holbrook) — This method is less expensive and as accurate as Folin's silver lactate method

(1) Principle Uric acid is precipitated in combination with zinc and is separated by centrifugation. Uric acid is set free by an acid chloride solution (HCl NaCl) and the rest of the estimation is the same as in the direct method

(2) Technic To exactly 5 cc of blood filtrate in a centrifuge tube add 2 cc of water, exactly 0.4 cc of 1 per cent zinc chloride solution, and 0.7 cc of 2 per cent sodium carbonate solution made from anhydrous sodium carbonate. Stir with a rod, then rinse the rod with a few drops of water. After standing 5 minutes, balance with another tube and centrifuge for 3 minutes. Discard the supernatant fluid. To the precipitate add 1 cc of Folin's hydrochloric acid sodium chloride reagent and stir with a rod. Dilute with 4 cc of water and stir until all is dissolved, rinse the rod. Drain the liquid into a test tube labelled 1, and add exactly 0.5 cc of modified standard uric acid solution. Into another test tube labelled 2, measure 4.5 cc of water and exactly 1 cc of modified standard uric acid solution. Into each tube put 3 drops of 20 per cent lithium sulphate, 2 cc of sodium cyanide and 1 cc of uric acid reagent, as above. Mix, after 2 minutes heat in the bath for 2 minutes cool, dilute to 25 cc and mix. Estimate with the colorimeter and calculate as in the direct estimation. If the estimation is high, it is not necessary to add uric acid to the unknown

(3) Reagents (a) Zinc chloride reagent Make this frequently by 1 in 10 dilution of a 10 per cent stock solution which has been allowed to settle clear

(b) Hydrochloric acid sodium chloride solution Dissolve 10 grams of sodium chloride in 100 cc of N/10 hydrochloric acid

4 **Blood Sugar** — The Shaffer-Hartman method as modified by Haskins and Holbrook is recommended

(a) *Modified Shaffer Hartman Method* — (1) Principle This is identical with that for the urine sugar method

(2) Technic Proceed exactly as directed for urine sugar on page 372 except that 5 cc of protein free filtrate is used instead of the dilute urine. Use the zinc filtrate on page 386 if true sugar values are desired

Calculation In Table 18 find the per cent of dextrose opposite the cc of sodium thiosulphate used for titration in column B

If the blood sugar content is very high, use 2.5 cc of filtrate and 2.5 cc of distilled water and multiply the per cent figure by 2

(b) *Folin's¹ Revised Colorimetric Method* — This is a satisfactory method but the blue color is not directly proportional to the concentration of dextrose so that errors are introduced if the concentration of dextrose in the blood differs widely from the concentration in the standard. This may be overcome by dilution

(1) Principle Copper sulphate in alkaline solution is reduced by dextrose and other reducing substances to cuprous oxide in quantitative amounts. This when

¹ Folin O. Two Revised Copper Methods for Blood Sugar Determination. J Biol Chem, 82: 83-93 (April) 1929

sulphuric acid (10 cc of C P acid plus 30 cc of water) and 20 cc of glacial acetic acid (99 per cent) Mix well Make fresh once a week The reagent will keep better if the sodium molybdate solution is treated with bromine before adding the acid solutions (see the original article)

(c) Stock solutions of dextrose (1) Dissolve exactly 5 gm of C P anhydrous dextrose (e g, Pfanstiehl's) in 500 cc of saturated benzoic acid solution (saturated benzoic acid is prepared by dissolving 2.5 grams in 1 liter of boiling distilled water) (2) Dilute exactly 20 cc of (1) to 100 cc in a volumetric flask with saturated benzoic acid solution Both stock solutions keep indefinitely Saturate with toluol

(d) Standard dextrose solutions (A) Dilute exactly 5 cc of stock solution (2) to 100 cc in a volumetric flask with distilled water (B) Dilute 5 cc of (2) to 50 cc in a volumetric flask with distilled water Add toluol to both A and B A contains 0.1 mg and B 0.2 mg per cc Prepare these dilute solutions each week

(c) *Folin's¹ Micro blood Sugar Method*—When it is impossible or inadvisable to draw venous blood as in children, nervous patients or those having small veins this method may be resorted to It can be used for sugar estimation on venous blood when there is a shortage of blood as the results are practically identical with those by the Shaffer Hartman or Folin Wu methods

After a fasting period, as before breakfast finger blood and venous blood show the same content of dextrose, at other times, particularly during periods of absorption of carbohydrates, the finger blood may have a higher content than venous blood If used for the dextrose tolerance test, the curves for normals and for diabetics need to be determined for finger blood samples, since these will undoubtedly be somewhat different from those for venous blood

Drawing the blood wash the finger with very warm water, wipe dry, and at once massage it with a stripping motion toward the tip, while the finger is well congested make a vigorous stab with the lancet and collect the blood in a watch glass Quickly measure the 0.1 cc required with the micropipette

(1) Principle The sugar is oxidized by alkaline potassium ferricyanide the latter being reduced to ferrocyanide The ferrocyanide is converted quantitatively into Prussian blue and this is estimated colorimetrically

(2) Technic First measure 5 cc of 0.4 per cent sodium tungstate and 5 cc of N/37.5 sulphuric acid into a 15 cc centrifuge tube Now measure accurately 0.1 cc of blood and deliver it to the lower part of the tube draw up clear liquid into the pipette twice, and finally blow through the pipette Stir the liquid with a rod but do not rinse it After 10 minutes' standing centrifugate for 3 to 5 minutes Decant the clear liquid into a clean dry test tube, there should be enough for 2 estimations

Prepare 2 test tubes Into A measure exactly 4 cc of the protein free blood extract, into B exactly 4 cc of dilute dextrose standard, add to both tubes exactly 1 cc (2 cc if a high estimation is expected) of ferricyanide reagent, 1 cc of sodium cyanide carbonate reagent and mix Plug the tubes with cotton and put them in a boiling water bath After 8 minutes, cool the tubes, add to each 3 cc of the acid ferric iron reagent, flowing it down the wall of the tube slowly to prevent excessive foaming and mix gently After 5 minutes transfer to 25 cc flasks or graduates rinse the tubes with water and dilute to the 25 cc mark and mix Compare in the

¹Folin, O A New Blood Sugar Method J Biol Chem 77 421-430 (May) 1928

Folin O The Micro Method for the Determination of Blood Sugar New England J Med 206 727-729 (April 7) 1932

colorimeter, setting the standard at 20 mm, make several readings. The readings should be between 13 and 30 mm for accurate estimations (see note 2)

$$\text{Calculation } \frac{\text{Mm standard}}{\text{Mm blood}} \times 100 = \text{mg of dextrose per 100 cc of blood}$$

Notes (1) The plungers and cups of the colorimeter must be very clean before being used. Use dilute sodium hydroxide occasionally for more effective cleaning. (2) If the blood gives too pale a color resulting in a reading above 30 mm, run another estimation, using a more dilute standard consisting of 2 cc of dilute dextrose plus 2 cc of water in tube B. Divide the calculated results by 2. If the blood gives a too intense blue color resulting in a reading below 13 mm, run another estimation, using 2 cc of the protein free fluid plus 2 cc of water without changing the standard. Multiply the calculated result by 2.

(3) The micropipette may be calibrated as described on page 329 or by filling to the mark with mercury and emptying into a weighed beaker. The weight of the mercury should be 1.355 grams at room temperature.

The pipette must be kept very clean. Wash it thoroughly as soon as possible after measuring the blood and use hot cleaning fluid in it frequently.

(4) Folin states that with low estimations the character of the color may be wrong because of the amount of yellow ferricyanide left unchanged. This may be compensated by adding ferricyanide reagent to the standard a drop at a time and comparing with the blood mixture in the graduate before dilution, but more than 0.1 cc of reagent should not be used.

(3) Reagents (a) 0.4 per cent sodium tungstate. Dilute 20 cc of 10 per cent sodium tungstate solution to 500 cc and add toluol.

(b) N/37.5 sulphuric acid. Dilute 20 cc of 3/4 normal sulphuric acid to 500 cc and add toluol.

Note. These two separate solutions seem better than the one mixed reagent suggested by Folin.

(c) Potassium ferricyanide reagent. Dissolve exactly 1 gram of C.P. potassium ferricyanide in water and dilute to 250 cc in a volumetric flask. Keep the solution in the dark in a brown bottle. The ferricyanide must be the purest obtainable (Merck's or Baker's is satisfactory) and must be free of ferrocyanide. Other samples of ferricyanide must be tested for the presence of ferrocyanide.

(d) Acid ferric iron solution. This contains a protective colloid to prevent precipitation of the Prussian blue. Put 5 grams of finely powdered Eimer and Amend's soluble gum ghatti in an Erlenmeyer flask, add 250 cc of distilled water, and shake occasionally. After 2 to 4 hours when most of the gum has dissolved strain through a cloth. Dissolve 1.25 grams of ferric sulphate ($7H_2O$) or 0.9 grams of the anhydrous ferric sulphate in a mixture of 25 cc of water and 19 cc of 85 per cent phosphoric acid by warming. Cool the solution and mix it with the gum ghatti. Disregard the slight turbidity. Now oxidize impurities by adding concentrated potassium permanganate solution a few drops at a time until the pink color remains for at least 10 minutes. Folin used 3.7 cc of 1 per cent potassium permanganate for 5 grams of gum ghatti. Keep in a 37° incubator for a few days until the turbidity has disappeared. When the reagent deteriorates so that the Prussian blue tends to precipitate during the colorimetric estimation make up a fresh batch of reagent.

(e) Sodium cyanide carbonate reagent Dissolve 4 grams of anhydrous sodium carbonate in 50 cc of distilled water in a 250 cc flask Dissolve 0.75 grams of sodium cyanide (Merck's) in 100 cc of water and add it to the carbonate Dilute to the mark and mix

(f) Dextrose solutions A Dissolve exactly 1 gram of C P anhydrous dextrose (e g, Pfanstiehl's) in exactly 500 cc of saturated benzoic acid solution (this is made by dissolving 2.5 grams in 1 liter of hot distilled water)

B Dilute dextrose standard Measure exactly 2.5 cc of A into a 500 cc. volumetric flask, add 50 cc of saturated benzoic acid solution and dilute to the mark with distilled water Keep the solutions in glass stoppered bottles Saturate them with toluol

5 Dextrose Tolerance Test (Janney and Isaacson¹)—This test is not indicated if the fasting blood sugar is above 150 mg per 100 cc

(a) *Principle*—An excess of dextrose is ingested when the stomach is empty and the changes in the blood sugar level are noted by frequent blood sugar estimations The time when dextrose appears in the urine is also noted to determine the renal threshold for dextrose If the dextrose is not being properly oxidized or if the renal threshold for dextrose is abnormal the type of curve will vary from the normal

(b) *Technic*—Allow no food after 7 P M In the morning draw blood for fasting blood sugar determination Have the patient take rapidly 1.75 grams per kilogram of body weight of pure dextrose (the commercial grade is not satisfactory) in a cooled 40 per cent solution to which the juice of one lemon has been added Have the patient void urine at once and test for reduction Then, if negative, test at 15 minute intervals till positive or till the end of the test is reached Estimate the blood sugar on blood drawn one half hour, one hour, and 2 hours after giving dextrose If the blood sugar is above 120 mg at the end of 2 hours, estimate again at the end of 3 hours

There are many modifications of this test Some use 50 or 100 grams of dextrose Brill² has suggested a test breakfast, containing carbohydrate, which is satisfactory The dextrose may be made into candy as suggested by Haskins, thus making it more palatable The time periods are also varied The important thing is to use the same technic on all patients so that one becomes familiar with the results by the technic used Delay in absorption may give rise to error, hence in cases with marked gastrointestinal disturbances the test should be deferred

¹ Janney N W and Isaacson, V I A Blood Sugar Tolerance Test Proc Soc Exp Biol and Med 15 15-16 (Nov 21) 1917

² Brill I C The Effect of a Normal Meal upon the Blood Sugar Level in Health and in Certain Conditions of Disease J Lab and Clin Med 8 727-731 (August) 1923

Normal findings The fasting blood sugar is normal. The highest blood sugar is at the end of the first half hour and is usually not over 150 mg and the blood sugar is back to normal at the end of two hours. Glycosuria does not occur unless the blood sugar reaches 125 to 225 mg (true sugar, 105 to 200 mg), in other words, the renal threshold is normal.

6 One Hour Two Dose Dextrose Tolerance Test (Exton and Rose as modified by Gould¹)—This test is not indicated if the fasting blood sugar is above 150 mg.

(a) *Principle*—The purpose is the same as in the previous test but this test has proved simpler and more efficient for the detection of diabetes mellitus.

(b) *Technic*—Have the patient come to the laboratory in the morning without eating breakfast. Dissolve 175 gm of dextrose per kilo of body weight in water to make a 40 per cent solution and add the juice of one lemon. Take a first specimen of blood and of urine and administer one half of the dextrose solution at once. Take another specimen of blood and urine in exactly 30 minutes and give the other half of the dextrose solution at once. Take another specimen of blood and urine one hour after the first specimen. Do quantitative sugar determinations by the modified Shaffer Hartman or Folin Wu technic on the blood specimens and test the urine specimens for reduction.

7 Blood Chlorides (Whitehorn²)—This method is applicable to filtrate of either whole blood or blood plasma.

(a) *Principle*—The chlorides are precipitated from the Folin blood filtrate by means of standard silver nitrate in the presence of nitric acid and the excess of silver is titrated with standard thiocyanate solution using ferric alum as an indicator.

(b) *Technic*—To exactly 10 cc of protein free filtrate add about 5 cc of C.P. nitric acid (chloride free) and exactly 5 cc of standard silver nitrate solution. After mixing let it stand 5 minutes or more until the precipitate clumps together. Then add about 0.3 grams of powdered ferric alum crystals and titrate with standard thiocyanate solution, cautiously, yet not too slowly, until a slight but definite reddish tinge is obtained and remains more than 15 seconds in spite of vigorous mixing.

¹ Gould S. E. The One Hour Two Dose Glucose Tolerance Test. *Am J Clin Path* 7: 474-481 (Nov) 1937.

Sweeney J. S., Murhead J. J. and Allday L. E. Observations on the One Hour Two Dose Dextrose Tolerance Test. *Am J Clin Path* 7: 482-489 (Nov) 1937.

² Whitehorn J. C. Simplified Method for the Determination of Chlorides in Blood or Plasma (Supplement to Folin's System of Blood Analysis). *J Biol Chem* 45: 449-460 (Feb) 1921.

Calculation (Five minus the cc used for titration) times 100¹ equals mg of chlorine and this figure times 1.65 equals mg of sodium chloride in 100 cc of blood. Results are usually reported as sodium chloride

(c) *Reagents*—(1) Standard silver nitrate solution. Dissolve 4.791 grams of C.P. silver nitrate in distilled water in a 1 liter volumetric flask and dilute to the mark. Mix and keep in a brown bottle.

(2) Standard thiocyanate solution. Dissolve 3 grams of potassium thiocyanate (2.5 grams of sodium thiocyanate or 2.4 grams of ammonium thiocyanate) in 1 liter of distilled water and mix. Titrate 5 cc portions of the silver nitrate solution (as above using nitric acid and ferric alum). Dilute the thiocyanate solution so that 1 cc equals 1 cc of silver nitrate solution.

Notes (1) Test the reagents for the presence of chlorine. None is present in C.P. sodium tungstate and nitric acid.

(2) Avoid excess of oxalate as anticoagulant as it will interfere with the ferric thiocyanate reaction.

F Determinations on Plasma or Serum—1. **Alkali Reserve Estimation**—This test, often called the carbon dioxide combining power of the plasma, is the most important test available for the detection of acidosis or alkalosis. The titration technic is so much simpler than the gasometric method that it is hard to understand why the latter is still used.

(a) *Titration Method* (Van Slyke² as modified by Haskins and Osgood³)—This is one of the simplest and most important of all blood chemistry determinations.

(1) **Principle**. The bicarbonate of 2 cc of plasma is decomposed by 5 cc of N/50 hydrochloric acid. The excess of acid is titrated with N/50 sodium hydroxide to a pH of 7.4. The cc of acid combined with the bicarbonate (cc of acid taken less cc of alkali used for titration) multiplied by 22.4, gives the alkali reserve figure.⁴

(2) **Technic**. Either blood plasma or serum may be used. Centrifugate the blood as soon as possible, draw off the plasma or serum and place it in the icebox in a tightly corked test tube unless the estimation is to be done immediately. Some draw the blood and keep it

¹ One cubic centimeter of standard silver nitrate solution equals 1 cc of standard thiocyanate solution and is equivalent to 1 mg of chlorine.

² Van Slyke, D. D., Stillman, E., and Cullen, G. E. *Studies of Acidosis VIII. A Method for Titrating the Bicarbonate Content of the Plasma*. J. Biol. Chem. 38: 167-188 (May) 1919.

³ Haskins, H. D., and Osgood, E. E. *Modifications of Van Slyke's Titration Method for Estimating the Alkali Reserve of Blood*. J. Lab. and Clin. Med. 6: 37-41 (October) 1920.

⁴ The alkali reserve figure is the cc of dry carbon dioxide (measured at 0° C and 760 mm) which can be held in chemical combination, excluding dissolved carbon dioxide, by 100 cc of plasma after exposure to an atmosphere containing 5.5 per cent carbon dioxide (alveolar air) at 20° C.

under a layer of mineral oil to lessen the change due to exposure to air. The plasma or serum keeps well in the icebox.

Measure exactly 2 cc of plasma into a Florence flask, not the titration flask. Add exactly 5 cc of N/50 hydrochloric acid and one drop of caprylic alcohol, then rotate the flask for 1 or 2 minutes so as to spread the mixture in a thin film on the wall. Pour the liquid into a small Pyrex flask of the same size and shape as that containing the standard and wash the rest into it using 3 rinsings totalling 20 cc of distilled water. Add 0.3 cc of indicator and titrate with carbonate free N/50 sodium hydroxide until the color matches the turbid standard, adding at the last, single 0.02 cc drops from a fine tip. Make the color comparison by reflected light with both flasks standing on a white surface. When an exact match is secured, read the burette. If the right end point has been reached, the addition of another drop will make the plasma mixture slightly too yellowish. Always carry the titration to this point. Check the alkali once a day by adding another 5 cc of N/50 hydrochloric acid to the titrated mixture and titrating back to the same endpoint. Wash the flask at once to prevent deposit of neutral red. Ammonia will remove such a deposit. Always keep a test tube over the top of the burette to exclude carbon dioxide.

Calculation Deduct the cc of sodium hydroxide used from the titration value of 5 cc of the N/50 hydrochloric acid, then multiply by 22.4. The result is the alkali reserve figure, which agrees within 1.5 points with the figure obtained by Van Slyke's gasometric method.

(3) Reagents (a) **Permanent standards** These may be purchased from the Shaw Surgical Company, Portland, Oregon, or they may be made as follows. Prepare buffer phosphate solution with a pH of 6.8, paranitrophenol solution and amaranth solution. The buffer phosphate solution is made by mixing equal volumes of exactly M/15 monohydrogen and dihydrogen phosphate. The paranitrophenol solution is made by dissolving 20 mg in 10 cc of 95 per cent alcohol and diluting with water to 100 cc. The amaranth solution is made by dissolving 8 mg in 100 cc of water. The buffer phosphate solution of a pH of 7.4 is made by adding 21.0 cc of M/15 dihydrogen sodium phosphate to 79.0 cc of M/15 monohydrogen phosphate. Place 60 cc of the buffer phosphate solution having a pH of 7.4 in a 120 cc Pyrex Erlenmeyer flask and add 0.6 cc of the neutral red solution. In a similar flask place 60 cc of buffer phosphate having a pH of 6.8, add 5.2 cc of the paranitrophenol solution and add the amaranth solution a little at a time until the color matches that in the first flask. With most lots of amaranth this requires 5.6 cc. Put 30 cc of this standard into each of two 120 cc Pyrex Erlenmeyer flasks and add 0.2 cc of 10 per cent thymol in chloroform to each. Keep one as a clear standard. To the other add 20 mg of corn starch powder, this is the turbid standard. Seal the corks with paraffin. They are permanent but do not expose them to strong sunlight.

(b) Neutral red Dissolve 65 mg (Kahlbaum or National Aniline Co) in 100 cc of 50 per cent alcohol

(c) N/50 hydrochloric acid Prepare once in 2 months by diluting 50 cc of exactly N/10 acid to 250 cc Add chloroform as a preservative Determine the titration value against the N/50 sodium hydroxide as follows To a mixture of 20 cc of water, 0.3 cc of neutral red, and 1 cc of 0.05 per cent disodium phosphate add N/50 hydrochloric acid a small drop at a time, until the color matches the clear standard Now add 5 cc of N/50 hydrochloric acid and titrate This titration should require 4.95 to 5.05 cc of N/50 sodium hydroxide A still better method of standardizing the alkali is to add the 5 cc of acid at the completion of a titration of plasma and titrate back to the same end point

(d) N/50 sodium hydroxide, free of carbonate Boil a liter of distilled water for 2 minutes, cork loosely and cool rapidly Add 1.2 cc of clear 65 per cent sodium hydroxide and mix well Keep the flask tightly corked Titrate 5 cc portions of N/50 hydrochloric acid as directed for titration value of hydrochloric acid Keep the burette covered with a test tube Dilute the sodium hydroxide with the proper amount of recently boiled water to make the titration just 5 cc Mix and recheck Put 25 cc portions into a large number of 50 cc Pyrex flasks, cork each at once and seal the corks with hot paraffin Use the sodium hydroxide from one flask for only one day For large laboratories it is more convenient to keep the N/50 sodium hydroxide in a large Pyrex flask fitted with a soda lime tube to prevent ingress of CO_2 and a siphon tube of Pyrex glass for filling the burette

(b) *Gasometric Method* (Van Slyke and Cullen¹) —This is still used by some but for clinical purposes the titration method is preferable The newer manometric method first described by Van Slyke and Neill² involves the use of a more expensive apparatus but gives more accurate results Manometric methods are available for a great many substances³ but they are too difficult for clinical use although of great value in research

(1) Principle Blood plasma is shaken with air the carbon dioxide tension of which approximates that of normal arterial blood by which treatment it combines with as much carbon dioxide as it is able to hold under normal tension A known quantity is then measured into the apparatus treated with acid and subjected to a partial vacuum The carbon dioxide is thus liberated and is measured after being placed under atmospheric pressure The volume corresponding to 100 cc of plasma is then calculated and corrected for physically dissolved gas

(2) Technic Centrifugate the oxalated blood as soon as possible and keep the plasma cold The estimation is more accurate if a layer of mineral oil protects the blood until the plasma is drawn off Put about 3 cc of plasma into a separating funnel Blow normal alveolar air (last $\frac{1}{3}$ of expired air) through a bottle filled with broken glass which is connected with the stem of the funnel and through the funnel while it is lying on the table Close the cock and cork the funnel Shake with a rotary motion for 2 minutes The plasma takes up carbon dioxide until it is

¹ Van Slyke D D and Cullen G E Studies on Acidosis The Bicarbonate Concentration of the Blood Plasma Its Significance and its Determination as a Measure of Acidosis *J Biol Chem* 30 289-368 (June) 1917

² Van Slyke D D and Neill J M The Determination of Gases in Blood and Other Solutions by Vacuum Extraction and Manometric Measurements *J Biol Chem* 61 523-574 (Sept) 1924

³ Peters J P and Van Slyke D D Quantitative Clinical Chemistry Vol II Williams and Wilkins Baltimore 1931

in equilibrium with the enclosed air which contains about 5.5 per cent carbon dioxide

The cup at the top of the Van Slyke apparatus must be free of acid and carbonate before the estimation is started. The mercury reservoir is set in the upper ring which is so placed that mercury fills the burette both passages in the cock and also the capillary tube at the base of the cup. Close the cock. Moisten the entire wall of the cup by flowing in 0.1 to 0.15 cc of 1 per cent carbonate free ammonium

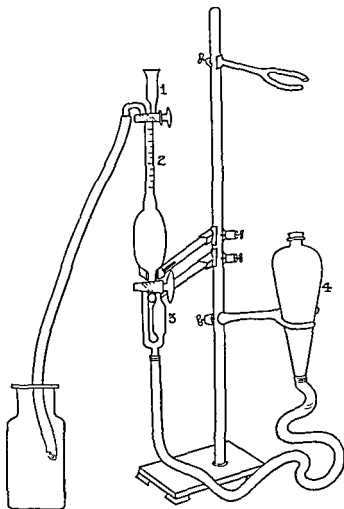


FIG. 15—Van Slyke apparatus. 1 Cup for introducing plasmas and reagents. 2 Microburette for measuring CO_2 . 3 Chamber for confining liquid while CO_2 is being measured. 4 Reservoir of mercury.

hydroxide and let it drain down. The plasma is to be delivered below this layer of ammonia.

Measure exactly 1 cc of plasma with a pipette that delivers 1 cc when drained between two marks into the cup, being sure to dip the tip below the ammonia before allowing the plasma to run out. Do not allow expired air to reach the cup. Lower the reservoir somewhat, open the cock cautiously and let the liquid flow slowly into the burette of the apparatus but leave the capillary full, and finally close the cock. Use about 0.5 cc of distilled water to rinse the cup, draw this into the

burette, and in a like manner use another 0.5 cc of water. Add a small drop of *caprylic alcohol* (0.02 cc is enough) and draw it just into the capillary so that it cannot float when the next solution is added. Add about 0.5 cc of 5 per cent *sulphuric acid*, and draw in enough to bring the mercury level about to the 2.5 cc mark. Put mercury in the cup and let it pass down to fill the capillary and the passage in the cock and then close the latter. Add to the cup a little ammonia and water, then draw off the liquid with a pipette.

Lower the reservoir enough to allow the mercury to sink just to the 50 cc mark and close the lower cock. Carefully lift the apparatus off the supports, and turn it upside down at least 15 times or connect it with the automatic shaker so that the carbon dioxide of the liquid and of the partial vacuum come to an equilibrium. Replace the apparatus, then lower the reservoir holding it in the hand until the level of the mercury is about 29 to 30 inches below the mercury level in the apparatus. Open the lower cock and adjust the reservoir so that the liquid passes slowly into the lower bulb. Close the cock when only a trace of liquid is left above it and no gas has passed into or below the cock.

Now turn the cock, so as to allow the mercury to pass by the left hand tube, raise the reservoir and hold it back of the microburette, so that the two mercury surfaces can be quickly brought to the same level. When levelled up close the lower cock and make the reading at the bottom of the meniscus of the aqueous liquid. With a magnifying glass exact readings can be made even when the meniscus comes between the marks. The column of liquid above the mercury should not be more than 0.1 cc. Note the temperature with a thermometer kept near the apparatus. Read the barometer. If more than 1 cc of gas is obtained as in alkalosis, repeat the estimation using 0.5 cc of plasma, in this case multiply the calculated results by 2.

Empty the apparatus as follows. Lower the mercury without opening the upper cock, turn the lower cock so that the aqueous liquid rises out of the bulb and floats on the mercury, then raise the reservoir and finally turn the upper cock so that the

TABLE 20—BAROMETRIC CORRECTION¹

Barometer		Factor for correction	Barometer		Factor for correction
Mm	Inches		Mm	Inches	
730	28.75	0.961	756	29.8	0.995
732	28.8	0.963	758	29.85	0.997
734	28.9	0.966	760	29.9	1.000
736	29.0	0.968	762	30.0	1.003
738	29.1	0.971	764	30.1	1.006
740	29.15	0.974	766	30.15	1.008
742	29.2	0.976	768	30.2	1.011
744	29.3	0.979	770	30.3	1.013
746	29.35	0.981	772	30.4	1.016
748	29.4	0.984	774	30.5	1.018
750	29.5	0.987	776	30.55	1.021
752	29.6	0.989	778	30.6	1.024
754	29.7	0.992	780	30.7	1.027

¹ Reproduced by permission from the J. Biol. Chem. 30: 289-346 (June) 1917

TABLE 21—CALCULATION OF THE ALKALI RESERVE¹

Cc of gas corrected for barometric pressure	Cc of CO ₂ bound as bicarbonate in 100 cc of plasma the temp of estimation being			
	15	20°	25	30
o 20	9 1	9 9	10 7	11 8
o 21	10 1	10 9	11 7	12 6
o 22	11 0	11 8	12 6	13 5
o 23	12 0	12 8	13 6	14 3
o 24	13 0	13 7	14 5	15 2
o 25	13 9	14 7	15 5	16 1
o 26	14 9	15 7	16 4	17 0
o 27	15 9	16 6	17 4	18 0
o 28	16 8	17 6	18 3	18 9
o 29	17 8	18 5	19 2	19 8
o 30	18 8	19 5	20 2	20 8
o 31	19 7	20 4	21 1	21 7
o 32	20 7	21 4	22 1	22 6
o 33	21 7	22 3	23 0	23 5
o 34	22 6	23 3	24 0	24 5
o 35	23 6	24 2	24 9	25 4
o 36	24 6	25 2	25 8	26 3
o 37	25 5	26 2	26 8	27 3
o 38	26 5	27 1	27 7	28 2
o 39	27 5	28 1	28 7	29 1
o 40	28 4	29 0	29 6	30 0
o 41	29 4	30 0	30 5	31 0
o 42	30 3	30 9	31 5	31 9
o 43	31 3	31 9	32 4	32 8
o 44	32 3	32 8	33 4	33 8
o 45	33 2	33 8	34 3	34 7
o 46	34 2	34 7	35 3	35 6
o 47	35 2	35 7	36 2	36 5
o 48	36 1	36 6	37 2	37 4
o 49	37 1	37 6	38 1	38 4
o 50	38 1	38 5	39 0	39 3
o 51	39 1	39 5	40 0	40 3
o 52	40 0	40 4	40 9	41 2
o 53	41 0	41 4	41 9	42 1
o 54	42 0	42 4	42 8	43 0
o 55	42 9	43 3	43 8	43 9
o 56	43 9	44 3	44 7	44 9
o 57	44 9	45 3	45 7	45 8
o 58	45 8	46 2	46 6	46 7
o 59	46 8	47 1	47 5	47 6
o 60	47 7	48 1	48 5	48 6
o 61	48 7	49 0	49 4	49 5
o 62	49 7	50 0	50 4	50 4
o 63	50 7	51 0	51 3	51 4
o 64	51 6	51 9	52 2	52 3
o 65	52 6	52 8	53 2	53 2

burette, and in a like manner use another 0.5 cc of water. Add a small drop of *caprylic alcohol* (0.02 cc is enough) and draw it just into the capillary so that it cannot float when the next solution is added. Add about 0.5 cc of 5 per cent *sulphuric acid*, and draw in enough to bring the mercury level about to the 2.5 cc mark. Put mercury in the cup and let it pass down to fill the capillary and the passage in the cock and then close the latter. Add to the cup a little ammonia and water, then draw off the liquid with a pipette.

Lower the reservoir enough to allow the mercury to sink just to the 50 cc mark and close the lower cock. Carefully lift the apparatus off the supports and turn it upside down at least 15 times or connect it with the automatic shaker so that the carbon dioxide of the liquid and of the partial vacuum come to an equilibrium. Replace the apparatus, then lower the reservoir holding it in the hand until the level of the mercury is about 29 to 30 inches below the mercury level in the apparatus. Open the lower cock and adjust the reservoir so that the liquid passes slowly into the lower bulb. Close the cock when only a trace of liquid is left above it and no gas has passed into or below the cock.

Now turn the cock, so as to allow the mercury to pass by the left hand tube raise the reservoir and hold it back of the microburette, so that the two mercury surfaces can be quickly brought to the same level. When levelled up close the lower cock and make the reading at the bottom of the meniscus of the aqueous liquid. With a magnifying glass exact readings can be made even when the meniscus comes between the marks. The column of liquid above the mercury should not be more than 0.1 cc. Note the temperature with a thermometer kept near the apparatus. Read the barometer. If more than 1 cc of gas is obtained as in alkalosis, repeat the estimation using 0.5 cc of plasma, in this case multiply the calculated results by 2.

Empty the apparatus as follows. Lower the mercury without opening the upper cock. turn the lower cock so that the aqueous liquid rises out of the bulb and floats on the mercury, then raise the reservoir and finally turn the upper cock so that the

TABLE 20.—BAROMETRIC CORRECTION¹

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738	29.1	0.971	764	30.1	1.006
740	29.15	0.974	766	30.15	1.008
742	29.2	0.976	768	30.2	1.011
744	29.3	0.979	770	30.3	1.013
746	29.35	0.981	772	30.4	1.016
748	29.4	0.984	774	30.5	1.018
750	29.5	0.987	776	30.55	1.021
752	29.6	0.989	778	30.6	1.024
754	29.7	0.992	780	30.7	1.027

¹ Reproduced by permission from the J. B. of Chem. 30: 289-346 (June) 1917

TABLE 21—CALCULATION OF THE ALKALI RESERVE¹

Cc of gas corrected for barometric pressure	Cc of CO ₂ bound as bicarbonate in 100 cc of plasma the temp of estimation being			
	15	20	25	30
o 20	9 1	9 9	10 7	11 8
o 21	10 1	10 9	11 7	12 6
o 22	11 0	11 8	12 6	13 5
o 23	12 0	12 8	13 6	14 3
o 24	13 0	13 7	14 5	15 2
o 25	13 9	14 7	15 5	16 1
o 26	14 9	15 7	16 4	17 0
o 27	15 9	16 6	17 4	18 0
o 28	16 8	17 6	18 3	18 9
o 29	17 8	18 5	19 2	19 8
o 30	18 8	19 5	20 2	20 8
o 31	19 7	20 4	21 1	21 7
o 32	20 7	21 4	22 1	22 6
o 33	21 7	22 3	23 0	23 5
o 34	22 6	23 3	24 0	24 5
o 35	23 6	24 2	24 9	25 4
o 36	24 6	25 2	25 8	26 3
o 37	25 5	26 2	26 8	27 3
o 38	26 5	27 1	27 7	28 2
o 39	27 5	28 1	28 7	29 1
o 40	28 4	29 0	29 6	30 0
o 41	29 4	30 0	30 5	31 0
o 42	30 3	30 9	31 5	31 9
o 43	31 3	31 9	32 4	32 8
o 44	32 3	32 8	33 4	33 8
o 45	33 2	33 8	34 3	34 7
o 46	34 2	34 7	35 3	35 6
o 47	35 2	35 7	36 2	36 5
o 48	36 1	36 6	37 2	37 4
o 49	37 1	37 6	38 1	38 4
o 50	38 1	38 5	39 0	39 3
o 51	39 1	39 5	40 0	40 3
o 52	40 0	40 4	40 9	41 2
o 53	41 0	41 4	41 9	42 1
o 54	42 0	42 4	42 8	43 0
o 55	42 9	43 3	43 8	43 9
o 56	43 9	44 3	44 7	44 9
o 57	44 9	45 3	45 7	45 8
o 58	45 8	46 2	46 6	46 7
o 59	46 8	47 1	47 5	47 6
o 60	47 7	48 1	48 5	48 6
o 61	48 7	49 0	49 4	49 5
o 62	49 7	50 0	50 4	50 4
o 63	50 7	51 0	51 3	51 4
o 64	51 6	51 9	52 2	52 3
o 65	52 6	52 8	53 2	53 2

TABLE 21—CALCULATION OF THE ALKALI RESERVE ¹—(Continued)

Cc of gas corrected for barometric pressure	Cc of CO ₂ bound as bicarbonate in 100 cc of plasma the temp of estimation being			
	15	20	25	30
o 66	53 6	53 8	54 1	54 1
o 67	54 5	54 8	55 1	55 1
o 68	55 5	55 7	56 0	56 0
o 69	56 5	56 7	57 0	57 0
o 70	57 4	57 6	57 9	57 9
o 71	58 4	58 6	58 9	58 8
o 72	59 4	59 5	59 8	59 7
o 73	60 3	60 5	60 7	60 6
o 74	61 3	61 4	61 7	61 6
o 75	62 3	62 4	62 6	62 5
o 76	63 2	63 3	63 6	63 4
o 77	64 2	64 3	64 5	64 3
o 78	65 2	65 3	65 5	65 3
o 79	66 1	66 2	66 4	66 2
o 80	67 1	67 2	67 3	67 1
o 81	68 1	68 1	68 3	68 0
o 82	69 0	69 1	69 2	69 0
o 83	70 0	70 0	70 2	69 9
o 84	71 0	71 0	71 1	70 8
o 85	71 9	72 0	72 1	71 8
o 86	72 9	72 9	73 0	72 7
o 87	73 9	73 9	74 0	73 6
o 88	74 8	74 8	74 9	74 5
o 89	75 8	75 8	75 8	75 4
o 90	76 8	76 7	76 8	76 4
o 91	77 8	77 7	77 7	77 3
o 92	78 7	78 6	78 7	78 2
o 93	79 7	79 6	79 6	79 2
o 94	80 7	80 5	80 6	80 1
o 95	81 6	81 5	81 5	81 0
o 96	82 6	82 5	82 4	82 0
o 97	83 6	83 4	83 4	82 9
o 98	84 5	84 4	84 3	83 8
o 99	85 5	85 3	85 2	84 8
1 00	86 5	86 2	86 2	85 7

¹ Reproduced by permission from Van Slyke D D and Cullen G E Studies of Acidosis I Bicarbonate Concentration of the Blood Plasma Its Significance and Its Determination as a Measure of Acidosis J Biol Chem 30 289-346 (Jun) 1917

liquid is forced out through the bent tube into the rubber tube which conveys it into a bottle Leave a mercury seal in the cock The next estimation may be made without further washing When all estimations are finished fill the apparatus between the cocks with water but do not draw in air at any time

Calculations Correct the observed volume of gas for barometric pressure by multiplying it by the factor given in Table 20 (p 400) In Table 21 find this

corrected volume in the left hand column and read the alkali reserve figure opposite it in the proper temperature column

2 Serum Calcium (Clark Collip¹ modification of the Kramer Tisdall² method)

(a) *Principle*—Calcium is precipitated directly from the serum as calcium oxalate which is recovered by centrifugation. Oxalic acid is liberated by the addition of sulphuric acid to the precipitate, and is titrated with standard potassium permanganate which oxidizes the oxalic acid

(b) *Technic*—The serum (oxalate or citrate plasma must not be used) must be centrifugated until absolutely free of suspended particles. The clear liquid can be drawn off without disturbing the sediment by using a pipette having a very fine tip with a rubber bulb attached

Measure into an absolutely clean centrifuge tube³ exactly 2 cc of the serum. Add 2 cc of redistilled water and 1 cc of 4 per cent ammonium oxalate. Mix well with a rod and rinse the rod with 3 drops of the oxalate solution. After it has stood at least 30 minutes (60 is better) centrifugate 5 minutes or until the liquid is quite clear and the precipitate is tightly packed. Discard the liquid and stand the tube upside down on clean filter paper draining it thus for 5 minutes. Wipe out with filter paper the liquid on the wall near the mouth of the tube.

Add 3 cc of dilute ammonium hydroxide stir up the precipitate and rinse the rod. Centrifugate decant and drain exactly as before. Another washing with dilute ammonia increases the accuracy. Add 2 cc of N/1 sulphuric acid by blowing it from the pipette so that the stream will stir up the precipitate. If necessary use a small rod but rinse it before removing it. Warm the tube in a Pyrex beaker of water. The bath is to be kept at 70 to 75° C until the titrations are finished. After 5 minutes warming the precipitate is dissolved and the oxalic acid that has been set free from the calcium oxalate may be titrated. Add slowly N/100 potassium permanganate from a micro burette⁴ having a fine tip. Keep the tube in the bath and shake it sufficiently to secure mixing. When near the end point, i.e. when the pink color disappears slowly use a rod so as to secure quick mixing to the very tip of the tube. A faint pink color must remain 1 minute after adding the last drop of permanganate. Read the burette to 0.01 cc. A control titration of 2 cc of the N/1 sulphuric acid should previously have been run.

Calculation Deduct the control for the sulphuric acid from the cc of permanganate used and multiply by 10 the result is milligrams of calcium in 100 cc of serum or plasma. 1 cc of permanganate is equivalent to 0.2 mg of calcium. The factor 10 is 50×0.2 since the 2 cc of plasma used is $\frac{1}{50}$ of 100 cc.

(c) *Reagents*—Prepare all reagents with water redistilled from permanganate. To the best available distilled water add potassium permanganate solution until

¹ Clark E. P. and Collip J. B. A Study of the Tisdall Method for the Determination of Blood Serum Calcium with a Suggested Modification. J. Biol. Chem. 63, 461-464 (March) 1925.

² Kramer B. and Tisdall F. F. A Simple Technic for the Determination of Calcium and Magnesium in Small Amounts of Serum. J. Biol. Chem. 47, 475-481 (Aug.) 1921.

³ Cleaning the centrifuge tube. Dichromate sulphuric acid mixture is specified for cleaning. There must be no grease on the wall to prevent perfect drainage and of course there must be no oxidizable substance attached to the wall.

⁴ Micro burette. A special 5 cc burette (marked for 0.02 cc) having a glass cock is obtainable. To fill it attach a rubber tube to the top and draw the permanganate in by suction. A thin film of vaseline may be applied to the outside of the tip so that smaller drops will be secured. The burette is suspended over the bath during the titration.

pinkish and redistill it after it has stood several days. N/1 sulphuric acid prepared with this water should give a control titration of not over 0.04 cc.

(1) Dilute ammonium hydroxide. Add 2 cc. of C P ammonium hydroxide to 98 cc. of redistilled water. The solution is better if saturated with calcium oxalate; this will become clear on standing.

(2) N/1 sulphuric acid (approximate). Mix 2.8 cc. of C P acid with 97 cc. of redistilled water.

(3) Potassium permanganate. Since N/100 permanganate weakens within a few days it is advisable to prepare it each day that estimations are made, by dilution of N/10 permanganate.

(a) Stock solution. Dissolve 0.35 gm. of potassium permanganate per 100 cc. in redistilled water to make a solution stronger than N/10. After it has stood several days use it for titrating 5 cc. portions of N/10 oxalic acid in the presence of 50 cc. of hot 10 per cent sulphuric acid. Then dilute the permanganate with redistilled water so that it becomes exactly N/10. This must be rechecked every few weeks (see (b) below).

(b) N/10 oxalic acid. Dry a gram or two of C P sodium oxalate in a drying oven at 100° for 3 or 4 hours. Cool in a desiccator. Dissolve exactly 0.67 gm. in about 30 cc. of water in a 100 cc. volumetric flask. Add 2.8 cc. of C P sulphuric acid cautiously while mixing. Cool and dilute to the mark. Prepare N/100 oxalic acid by diluting 5 cc. of N/10 to 50 cc. with redistilled water and use to check N/100 permanganate; this dilute solution must be prepared fresh each day that it is used.

(c) N/100 permanganate. Dilute exactly 5 cc. of the N/10 stock solution to 50 cc. with redistilled water. This should be checked against N/100 oxalic acid. Titrate 2 cc. of the oxalic acid plus 2 cc. of N/1 sulphuric acid exactly as in the calcium estimation and deduct the control from the micro burette reading.

When, after some weeks, it is found that the N/10 permanganate is a little weak, it is necessary to use more than 5 cc. for preparing 50 cc. of N/100 solution. For example, if 10.2 cc. of stock permanganate is required to titrate 10 cc. of exact N/10 oxalic acid, then 5.1 cc. of the solution must be used to prepare 50 cc. of N/100 permanganate.

3. **Blood Phosphate (Benedict and Theis' method¹)**—The inorganic phosphate in serum or plasma can be easily estimated colorimetrically.

(a) *Principle*—Protein is precipitated with trichloroacetic acid. The filtrate contains the inorganic phosphate in the form of phosphoric acid. When molybdic acid is reduced by hydroquinone in the presence of phosphoric acid, a deep blue color is developed which is proportional to the amount of phosphorus present. Organic phosphorus compounds do not interfere because none are present in the filtrate (Benedict).

(b) *Technic*—Start the estimation as soon as possible. Badly hemolyzed blood should not be used. Measure exactly 2 cc. of plasma or serum into a centrifuge tube, add 4 cc. of distilled water and 4 cc. of 20 per cent trichloroacetic acid solution. A lower concentration of the acid is unsatisfactory. Shake and let the tube stand at least 10 minutes. The plasma and acid may be mixed best in a small beaker.

¹ Benedict S. R. and Theis R. C. A Modification of the Molybdic Method for the Determination of Inorganic Phosphorus in Serum. J. Biol. Chem. 61: 63-71 (August) 1924.

and then be transferred to the centrifuge tube. Centrifugate about 10 minutes pour the supernatant fluid onto a 7 cm ash free filter, enough filtrate will be secured for two estimations. If desired the tube may be corked and the determination be made later even the next day.

Measure exactly 3 cc of clear filtrate into an 8 by 1 inch test tube. Into another tube measure exactly 3 cc of dilute phosphate standard solution. To each add 5 cc of distilled water, 1 cc of hydroquinone reagent, and 1 cc of molybdic acid reagent and mix well. Stopper the tubes with corks having a small groove cut on the side as a vent, and heat in a boiling water bath for 10 minutes. There is no loss in volume in the tubes. Cool to room temperature and compare in a colorimeter setting the standard at 15 mm. The blue color is permanent for at least one hour.

$$\text{Calculation } \frac{62.5^*}{\text{Reading of the blood}} = \text{mg of phosphorus in 100 cc of plasma}$$

(c) *Reagents*—(1) Trichloroacetic acid solution. Use the purest acid obtainable. Dissolve 40 grams of colorless crystals in enough water to make 200 cc of solution.

(2) Benedict's hydroquinone reagent. Dissolve 1 gram of hydroquinone and 30 grams of sodium bisulphite in water and dilute to 200 cc. This solution keeps well.

(3) Benedict's molybdic acid reagent. Dissolve 20 grams of C P molybdic acid ('special' 99.9 per cent) in 50 cc of 10 per cent sodium hydroxide, warming gently. Cool, dilute to 200 cc and filter if not clear. Transfer to a large flask and add C P sulphuric acid in small portions until 200 cc has been added with frequent shaking and cooling under the cold water tap. The reagent becomes deep blue on standing but this is not apparent when 1 cc is diluted with the reaction mixture in an estimation.

(4) Standard phosphate solutions. (a) A strong stock solution is prepared by dissolving 0.11 grams of C P anhydrous acid potassium phosphate (KH_2PO_4 , Merck's reagent 'Sorensen's potassium phosphate' is the purest) in distilled water and diluting to exactly 300 cc. Add chloroform and keep the bottle tightly corked.

(b) Dilute standard. Dilute 10 cc of the stock solution to 100 cc when needed, 3 cc of this contains 0.025 mg of phosphorus.

4. Estimation of Blood Proteins¹ (Wu and Ling).—(a) *Principle*.—A reagent is used which develops a blue color with phenols. Tyrosin in the protein that is being estimated reacts to give the color and this is compared colorimetrically with the color given by a standard of pure tyrosin that has been treated with the reagent simultaneously.

Wu's latest figures correspond closely to the relative content of tyrosin in pure proteins of human blood.

(b) *Technic*.—Fasting blood should be used (no free amino acids). Draw about 10 cc. Quickly put 5 cc into a test tube containing 10 mg of dry potassium oxalate and the rest into a centrifuge tube. When the latter has clotted firmly

* $62.5 = 15 \text{ (mm of standard)} \times 0.025 \text{ (mg of phosphorus in the standard)} \times 100$ divided by 0.6 (cc of plasma taken for estimation)

¹ See page 519 for a simple but somewhat less accurate method.

(a half hour to several hours), centrifugate both samples until clear. Start the albumin and fibrinogen estimations as soon as possible.

(1) **Albumin Estimation** Measure exactly 1 cc of fresh hemoglobin free serum or plasma into a test tube. Add exactly 3 cc of water and 6 cc of 48 per cent ammonium sulphate solution. Mix well. Let stand 1 hour or longer. If the protein content is low (mixture is less opaque) let stand 2 to 4 hours or use 2 cc of serum or plasma and 2 cc of water for estimation.

Filter through a double thickness of good filter paper such as Whatman No. 2, 9 cm in diameter. If the filtrate is turbid, turn back on to the same filter, and repeat until a clear filtrate is secured.

Measure exactly 1 cc of filtrate into a centrifuge tube. Add 10 cc of water and 1 cc of 10 per cent sodium tungstate. Mix with a slender glass rod. Add 1 cc of $\frac{3}{4}$ N sulphuric acid and stir with the same rod. Rinse the rod with a few drops of water before removing it.

After 5 to 10 minutes, centrifugate, then decant the clear liquid as completely as possible from the packed precipitate. Redissolve and reprecipitate as follows: to the precipitate add 1 cc of sodium tungstate solution and stir until the precipitate has dissolved. Add 10 cc of water and mix, then 1 cc of $\frac{3}{4}$ N sulphuric acid, stir and rinse the rod. Let stand 5 to 10 minutes and centrifugate. Decant the liquid completely.

To the precipitate add 10 cc of water and 1 drop of 20 per cent sodium carbonate. Stir until dissolved. If necessary, use an extra drop of carbonate but never more than 2. When dissolved rinse the rod and transfer the liquid to an accurate 25 cc graduate or flask. Use 3 cc of water twice to rinse the tube into the graduate. In another 25 cc graduate prepare the standard as directed below. Add 0.5 cc of phenol reagent to each graduate then add to each 1 cc of 10 per cent sodium hydroxide practically simultaneously and mix with gentle rotation. Note the time. Dilute to exactly 25 cc and mix. Let stand *exactly* 1 hour, read in the colorimeter setting the standard at 20 mm. Finish the estimation within 10 minutes after the 1 hour period. After that time the colors in the protein and in the tyrosin mixtures change at different rates (Wu).

The Standard Used for All the Protein Estimations Measure exactly 1 cc of tyrosin solution into a 25 cc graduate. Add 0.5 cc of phenol reagent dilute to about 20 cc and mix. Add 1 cc of 10 per cent sodium hydroxide and mix gently. Note the time. Dilute to exactly 25 cc. Use for estimation after exactly 1 hour. In some cases it is desirable to have another standard of double strength (2 cc of tyrosin).

Calculation 1 mg tyrosin is equivalent to 21.4 mg albumin $\frac{20}{\text{mm unknown}} \times 0.2 \times 1000 = \text{mg tyrosin equivalent to the albumin of 100 cc serum or plasma}$
 $\text{Mg tyrosin} \times \frac{21.4}{1000} = \text{gm albumin per 100 cc or per cent}$ If 2 cc of serum was used for estimation divide the calculated result by 2.

(2) **Fibrinogen Estimated as Fibrin** The fibrin should be precipitated from oxalated plasma as soon as possible after the blood is drawn. Measure exactly 1 cc of plasma into a small Erlenmeyer flask add 28 cc of 0.8 per cent sodium chloride and 0.5 cc of 5 per cent calcium chloride. Mix and let stand undisturbed for 30 minutes or until a solid jelly has formed. Using a small glass rod with a tapered end twirl it gently in the jelly until all the fibrin is wrapped about the

rod If necessary, press the rod against the side of the flask while rotating it in order to squeeze the liquid out of the jelly Push the fibrin off the rod and press it lightly between dry filter paper to remove as much liquid as possible

Put the fibrin into a centrifuge tube, add 4 cc of 1 per cent sodium hydroxide, warm it in a beaker of water at 65 to 80° C and stir occasionally until the mass is disintegrated (the solution is turbid) Add 8 cc of water mix rinse the rod, and centrifugate Decant the clear liquid completely into a 25 cc graduate Cool to room temperature and add 1 cc of 5 per cent sulphuric acid Add 0.5 cc of phenol reagent, dilute to about 20 cc add 1 cc of 10 per cent sodium hydroxide and mix gently Note the time Dilute to 25 cc and let stand exactly 1 hour Read quickly in a colorimeter against the standard prepared as above, set at 20 mm

Calculation 1 mg tyrosin is equivalent to 11.3 mg fibrin

$$\frac{20}{\text{mm unknown}} \times 0.226 = \text{per cent fibrin}$$

$$\text{i.e. } \frac{20}{\text{mm unknown}} \times 0.2 \times 100 = \text{mg tyrosin equivalent to fibrin, mg tyrosin} \\ \times \frac{11.3}{1000} = \text{gm fibrin per 100 cc plasma}$$

(3) Globulin Estimation This is estimated by determining the total protein of serum and deducting the albumin The serum is obtained from fasting blood and must be free of hemoglobin

Dilute exactly 1 cc of clear serum with 9 cc of 0.8 per cent sodium chloride or 0.5 cc with 4.5 cc and mix Measure 1 cc of this or 3 cc of liquid from which the fibrinogen has been removed if there is a shortage of serum into a 25 cc graduate and add about 18 cc of water When the standard is ready for simultaneous treatment with reagents add 0.5 cc of phenol reagent and 1 cc of 10 per cent sodium hydroxide to the serum mixture Mix gently Note the time Dilute to 25 cc and mix Let stand exactly 1 hour and read quickly in the colorimeter against the standard set at 20 mm

Calculation 1 mg tyrosin is equivalent to 15.8 mg globulin

$$\frac{20}{\text{mm unknown}} \times 0.2 \times 1000 = \text{mg tyrosin equivalent to albumin plus globulin} \\ \text{in 100 cc of serum Deduct from the mg tyrosin equivalent of albumin plus globulin the mg tyrosin equivalent to albumin (see above) the result being the mg tyrosin equivalent to globulin Globulin tyrosin} \times \frac{15.8}{1000} = \text{gm globulin per 100 cc of serum}$$

All three estimations may be made with the aid of a single standard by bringing the 3 protein mixtures and the standard to the same stage in the graduates and then adding the phenol reagent and sodium hydroxide quickly to each

This method can be adapted to estimation of albumin and globulin in cerebrospinal fluid, in pleural and ascitic fluids and in urine if Bence Jones protein is absent

(c) Reagents—(1) Tyrosin solution dissolve 50 mg of Pfanstiehl C P tyrosin in 250 cc of N/10 hydrochloric acid and add chloroform or toluol (thymol gives a blue color with the reagent) this contains 0.2 mg of tyrosin per cc

(2) Ammonium sulphate solution dissolve 48 gm C P crystals in 70 cc of water and dilute to 100 cc Six cc of this solution contains as much sulphate as

5 cc of saturated solution The saturated solution is a troublesome reagent since crystals separate out whenever the temperature of the room is lowered

(3) Sodium tungstate and 35N sulphuric acid these are the same solutions as are used for the protein free filtrate

(4) Sodium carbonate solution Dissolve 10 gm of anhydrous sodium carbonate in 40 cc of distilled water and dilute to 50 cc

(5) Sodium hydroxide solutions 10 per cent, prepare a solution stronger than 10 per cent from 60 to 65 per cent sodium hydroxide solution titrate against N/1 sulphuric acid and dilute so that 1 cc will neutralize 2.5 cc of N/1 acid

One per cent, dilute 10 cc of 10 per cent to 100 cc

(6) Sulphuric acid, 5 per cent dilute 10 cc of C P sulphuric acid to 200 cc

(7) Sodium chloride, 0.8 per cent dissolve 8.0 gm of C P sodium chloride in 1000 cc of water

(8) Calcium chloride 5 per cent dissolve 5.0 gm of dry C P calcium chloride (weigh quickly) in 100 cc of water and filter

(9) Phenol reagent (Folin Ciocalteu) transfer 100 gm of sodium tungstate and 25 gm of sodium molybdate together with 700 cc of water to a 1500 cc Florence flask Add 50 cc of 85 per cent phosphoric acid and 100 cc of C P hydrochloric acid Connect to a reflux condenser by means of a cork or rubber stopper wrapped in tin foil, and boil gently for 10 hours At the end of the boiling period, add 150 gra of lithium sulphate, 50 cc of water, and a few drops of liquid bromine (caution!) Boil the mixture without the condenser for about 15 minutes to remove the excess bromine Cool, dilute to 1 liter, and filter The finished reagent should have no greenish tint, as this means the presence of blue reduction products which will lessen the range of true proportionality between different small amounts of tyrosin The reagent should be kept well protected against dust as organic materials will gradually produce slight reduction

5 Cholesterol (Myers and Wardell,¹ slightly modified) —This is estimated in oxalated whole blood or plasma by a colorimetric method

(a) *Principle* —Dried blood uniformly spread in a thin layer on fine particles of plaster is extracted with chloroform The chloroform solution of cholesterol is treated with sulphuric acid and acetic anhydride (Liebermann Burchard reaction) developing a bluish green color which is proportional to the amount of cholesterol present

Since moisture interferes with the reaction the chloroform and the reagents must be as nearly anhydrous as possible the pipettes colorimeter cups and other glassware must be dry

(b) *Technic* —The blood may be kept in the ice box half a day before starting the estimation Spread about 6 grams of plaster of Paris of good quality in a uniform layer in an evaporating dish about 4 inches in diameter Deliver exactly 1 cc of well mixed oxalated blood or plasma by drops all over the surface of the plaster * In about 5 minutes stir with a small rod until the plaster is uniformly colored and leave the rod in the dish When partly air dried the small lumps may

¹ Myers V C and Wardell E I The Colorimetric Estimation of Cholesterol in Blood Jour Biol Chem 36 147 156 (Oct) 1918

* Instead of spreading the blood on plaster of Paris it is more convenient to drop it on 2 or more pieces of filter paper and dry for an hour or more in a 35 to 40 incubator (Kamlet) Then fold the filter papers and place them in the Soxhlet extraction apparatus and proceed as directed in the original method No extraction shell is necessary Direct extraction as suggested by Kamlet J Simplified Micro-determination of Cholesterol in

be reduced by using the bottom of a test tube as a pestle. Scrape off the adhering plaster with a spatula. The blood plaster mixture, spread well on the bottom and up the wall of the dish, is dried in an oven at 90°C (see note 2) for 2 hours. After 1 hour of heating take the dish from the oven and stir up the powder then reheat. If there are any lumps break them up with the pestle. After complete drying, transfer the powder to a fat free paper extraction shell as completely as possible by the following method. Support the shell in a short cylinder and use a dry short stemmed funnel. Stand this cylinder on a sheet of glazed black paper. Pour the powder slowly with the aid of the rod through the funnel. Scrape off the rod. Scrape with a spatula the wall of the dish standing it on the black paper, until all substance seems to be loose and transfer to the funnel. Use a camel's hair brush to transfer the last of the powder from the dish, the spatula and the black paper, and finally from the funnel itself to the extraction shell. There must be no powder on the outside of the shell. Place the shell carefully in the extraction apparatus in such a position that the condensed chloroform will drip directly onto the powder. Use 20 cc of anhydrous chloroform in the small extraction flask. Heat with an electric heater adjusted so that there is active boiling and a constant drip of chloroform from the shell for 45 minutes. When the apparatus is cool, remove the flask. Pour the chloroform through a dry funnel into a dry accurate 25 cc measuring flask. Use several small portions of redistilled chloroform to rinse the extraction flask and the funnel. When the chloroform is at room temperature, it may be filled to the mark, mixed and estimated. The estimation may be postponed to the next day if the flask is corked and set in a dark place.

Exactly 5 cc is measured into a dry test tube. Into another tube measure 5 cc of dilute cholesterol standard solution. To each add exactly 0.2 cc (within 0.01 cc) of C P sulphuric acid and 2 cc of pure acetic anhydride. Mix well, cork loosely, place immediately in a beaker of water that is at 25°C and set in a dark place. After exactly 10 minutes compare in a colorimeter and secure the readings as soon as possible since the color changes. Chill the liquids in a bath of ice water before putting them into the colorimeter as this stabilizes the typical bluish green color. Set the standard at 15 or 20 mm and use a ground glass screen in the path of the light. Make several readings and average them. At ordinary temperature the color changes rapidly losing the blue tinge and becoming yellowish.

Calculation $\frac{(15 \text{ or } 20)}{\text{Reading of the blood}} \times 200^* = \text{mg of cholesterol in 100 cc of blood}$

Notes 1. An aqueous solution of naphthol green B has been suggested as an artificial standard but has not a sufficiently bluish tinge. A better standard is prepared as follows. Dilute 2.2 cc of 0.1 per cent aqueous solution of naphthol green B and 0.16 cc of 0.1 per cent methylthionine chloride (methylene blue) to 100 cc. Satisfactory dyes are obtainable from the National Aniline and Chemical Co. Set this as the standard at 23 mm the color is very close to that of the purest obtainable cholesterol set at 20 mm. The estimations of blood are the same as with pure cholesterol standard. Use 20 in the formula for calculation. This dilute standard must be made up each day. The concentrated dye solutions keep for over a month.

Whole Blood Serum and Plasma J Lab and Clin Med 19 883 (May) 1934 gave low estimations

200 equals 0.4 (mg cholesterol in standard) $\times \frac{25}{5} \times 100$

in the dark, but deteriorate later. Others using this artificial standard should test it against very pure cholesterol.

2 Overheating or too prolonged heating of the blood plaster mixture gives an extract which does not develop the typical bluish green color but a more yellowish color which is very difficult to match. By keeping the oven temperature down to 90° C, the extracts give almost exactly the same color as the pure cholesterol standard.

(c) *Apparatus*—Test tubes, pipettes and colorimeter. The extraction apparatus a Soxhlet or other similar syphon type of apparatus is not necessary or desirable. A simple form of fat extraction tube (Knorr) is quite suitable, a piece of bent glass rod is inserted to prevent the shell from blocking the bottom of the chamber. A reflux condenser is attached to the top of the tube by a cork stopper. A small extraction flask is attached below by means of a cork.

(d) *Reagents*—(1) Cholesterol solutions. Stock solution. Dissolve 80 mg of the purest cholesterol obtainable that is *free of ester* (Pfanstiehl's C P cholesterol is satisfactory) in 100 cc of anhydrous chloroform.

Dilute standard. Dilute exactly 5 cc of the stock solution to 50 cc with chloroform as needed and mix well. Keep these chloroform solutions in a cool dark place and tightly corked.

(2) Anhydrous chloroform. Treat 500 cc of C P chloroform with dry calcium chloride for several days with occasional shaking. Filter into a dry distilling flask and distill. Keep tightly corked.

(3) Sulphuric acid. Use the best grade of C P acid.

(4) Acetic anhydride. Use the best obtainable. Merck's blue label reagent is recommended and Pfanstiehl's is satisfactory.

6 **Bile Pigment in Blood**—(a) *Icterus Index*—This was first described by Meulengracht,¹ several modifications have been suggested. It is preferable to the more complicated and less accurate method of van den Bergh.

(1) *Principle*. Increases in bilirubin alter the intensity of the yellow color of blood plasma. These alterations are quantitatively determined by comparing the clear plasma or serum with standard potassium dichromate solution.

(2) *Technic*. Serum is preferable to plasma. Clinical carotinemia may be associated with enough carotin in the blood serum to change the icterus index 1 or 2 points but is not likely to introduce clinical error. White and Gordon² have developed a method for the determination of carotin.

Draw the blood after a fasting period and centrifugate. Perfectly clear plasma or serum is transferred to the cup of a colorimeter and readings are made against the dilute standard dichromate solution set at 15 or 20 mm.

¹ Meulengracht E. Bile Pigment in Blood Serum. *Deutsch Arch f klin Med* 132: 285 (July) 1920.

² White F D and Gordon Ethel M. The Estimation of the Serum Carotin. *J Lab and Clin Med* 17: 53-59 (Oct) 1931.

$$\text{Calculation } \frac{M_m \text{ standard}}{M_m \text{ unknown}} = \text{Icterus index}$$

If the plasma is highly colored, make an exact dilution with 0.9 per cent sodium chloride solution before estimation. Multiply the calculated index by the factor for dilution.

If the serum contains hemoglobin or is turbid, use the acetone technic of Newburger.¹ To 3 cc of serum or plasma in a graduated centrifuge tube add an equal volume of redistilled acetone. Mix, let it stand 5 minutes and centrifugate. Transfer the clear supernatant fluid to the colorimeter cup and proceed as above but multiply the results by 2, or still better, use the clear fluid in the Farahaugh and Medes method as described below and multiply the results by 2. If dilution is required, use the acetone instead of 0.9 per cent saline.

(3) Reagents (a) Prepare an exact 1 per cent solution of potassium dichromate as a stock solution.

(b) Dilute standard. Make this frequently by diluting exactly 1 cc of the 1 per cent solution to 100 cc with distilled water. If a few drops of C P sulphuric acid are added to this standard and it is kept in a brown bottle it will keep several months.

It is even more satisfactory to compare the serum or plasma in a biocolorimeter with the permanent standards of Farahaugh and Medes.² If the serum is too deeply colored to match the standard make a 1 to 10 dilution with 0.9 per cent saline and multiply the value on the nearest standard tube by 10. In some instances a 1 to 20 dilution will be necessary.

Preparation of the standards.—The stock solution is prepared by dissolving 0.5 grams of potassium dichromate in a 500 cc flask with water containing a few drops of sulphuric acid and diluting to the mark. Use water containing sulphuric acid in making the dilute standards also.

Label Standard	Stock Solution Cc	Water Cc
1	1.0	9.0
2	2.0	8.0
2.5	2.5	7.5
3	3.0	7.0
3.5	3.5	6.5
4	4.0	6.0
4.5	4.5	5.5
5	5.0	5.0
6	6.0	4.0
8	8.0	2.0
10	10.0	0.0

¹ Newburger R. A. Determination of the Icteric Index by the Acetone Method. J. Lab. & Clin. Med. 22: 1192-1195 (Aug.) 1937.

² Farahaugh C. C. and Medes G. A New Set of Potassium Dichromate Standards for Determination of the Icterus Index. J. Lab. and Clin. Med. 14: 681-682 (April) 1929.

Select 11 test tubes of uniform diameter and measure into them the quantities indicated in the table, labelling them with the icterus index value in the first column of the table. Cork, seal with paraffin, and keep in the dark when not in use.

(b) *Van den Bergh's Test*—This test for bilirubin in blood serum or plasma is recommended as a qualitative test to differentiate obstructive from hematogenous jaundice.

A quantitative estimation of bilirubin is possible but as it is much more difficult and much less accurate than the icterus index determination the latter is recommended.

(1) Principle Serum or plasma is treated with a diazotizing reagent and the red color which results from its reaction with bilirubin is taken as an index of the type and extent of bilirubinemia depending on the rate of appearance and depth of color.

(2) Technic (a) Direct reaction Mix 1 cc of clear serum or plasma and 1 cc of diazo reagent. A purplish red color appearing in 30 seconds is an immediate direct reaction, if it appears after 30 seconds, it is a delayed direct reaction, and if it appears first within 30 seconds but gradually becomes more intense afterward, it is a biphasic reaction.

(b) Indirect reaction¹ In a centrifuge tube mix 1 cc of serum or plasma with 0.5 cc of reagent. Let it stand 15 minutes and add 2.5 cc of 95 per cent alcohol and 1 cc of saturated ammonium sulphate solution. Mix after each addition and centrifuge. Compare the clear supernatant fluid in the colorimeter against the cobalt standard.²

$$\text{Calculation } \frac{\text{Mm standard}}{\text{Mm unknown}} \times 4 \times 0.4 = \text{mg of bilirubin per 100 cc}$$

One unit is 5 mg of bilirubin per 1000 cc of blood. Express the results in mg per 100 cc.

(3) Reagents (a) Dissolve 10 gram of sulphanilic acid in dilute hydrochloric acid (150 cc of C P hydrochloric acid in 1000 cc of water). This keeps well.

(b) 0.4 per cent sodium nitrite, freshly prepared.

(c) 6 gm of disodium hydrogen phosphate (Na_2HPO_4) dissolved and diluted to 100 cc.

(d) Diazo reagent 25 parts of reagent (a) plus 1 part of reagent (b). Just before using mix equal parts of this mixture and (c). This reagent does not keep well and should be made fresh each day.

(e) Standard Dissolve 13 grams of anhydrous cobalt sulphate in 50 cc of distilled water in a 100 cc flask, add 40 cc of C P hydrochloric acid, cool and dilute to the mark. It is permanent if kept in the dark.

7 *Dye Tests for Liver Function*—Many such tests have been proposed, but the bromsulphalein test of Rosenthal⁴ seems to be the most satisfactory.

¹ Van den Bergh A A H. Diazo Test for Bilirubin in Blood. *Presse Medical* 29 441 (June 4) 1921.

² White F D. On Serum Bilirubin. I. The Diazo Reaction as a Quantitative Procedure. *British J Exper Path* 13 76-85 1932.

³ This standard was suggested by McNee as a substitute for the original ethereal solution of ferric thiocyanate.

⁴ Rosenthal S M and White E C. Clinical Application of the Bromsulphalein Test for Hepatic Function. *J A M A* 84 1112-1114 (April) 1925.

(a) *Principle*—A definite quantity of the dye is injected intravenously and the amount still remaining in the blood stream after 30 minutes is determined. Bromsulphalein is excreted chiefly in the bile hence, if liver function is impaired, it will remain longer in the blood stream and in higher concentration than in normal individuals.

(b) *Technic*—Two mg of bromsulphalein per kilogram of body weight is injected intravenously as a 5 per cent solution in sterile physiologic saline solution. The patient's weight in pounds divided by 55 gives the cc of 5 per cent solution to inject. Blood is withdrawn from the opposite arm 30 minutes after injection, the serum is separated by centrifugation and the per cent of dye in the serum is estimated by comparison with standards¹ in the comparator accompanying them after the addition of one or two drops of 10 per cent sodium hydroxide to bring out the color. A set of standards for use with a biocolorimeter may be made. Four mg in 100 cc of 0.2 per cent sodium hydroxide solution equals the 100 per cent standard.

Rosenthal² perfected the phenoltetrachlorophthalein test in 1922 but he and others later discovered several objections to this dye among which are the dangers of thrombosis of the vein after injection and the large amount of dye necessary.

8 *Congo Red Test*—(a) *For Determination of the Total Volume of the Circulating Blood* (Keith Rowntree and Geraghty³ as modified by Harris⁴)—(1) *Principle*. A known amount of dye is injected intravenously after removing blood for a standard. After allowing adequate time for thorough admixture with the dye blood is withdrawn from the opposite arm and the color of the plasma is compared with that of a standard. From the amount of dilution of the dye indicated, the total volume of the plasma and whole blood may be calculated.

(2) *Technic*. Dissolve 375 mg of Congo red in 25 cc of redistilled water to make a 15 per cent solution. Filter and sterilize by boiling or better, by autoclaving. As soon as it is cooled to body temperature do a venipuncture. Withdraw 5 cc of blood for a standard and then inject the number of cc of dye solution corresponding to one fourth the patient's body weight in kilos (i.e., a patient of 60 kilograms body weight would receive 15 cc). Not more than 18 cc should be injected in any case. After allowing at least 3 minutes never over 6 for mixing a second sample of blood is withdrawn from the opposite arm and mixed with oxalate to prevent clotting. Both samples of oxalated blood are centrifugated as for a volume index determination p. 437 and the volume of plasma per 100 cc of blood is calculated from the average of the two results. A standard is prepared from the plasma of the first sample of blood as follows: 2 cc of plasma 2 cc of a 1 to 100 dilution of the 15 per cent dye solution, and 4 cc. of 0.9 per cent sodium chloride solution, this is placed in the colorimeter and set at 10 mm. Then the dye content of the plasma of the second sample of blood after the addition of 6 cc of 0.9 per cent sodium chloride solution to 2 cc of the plasma is determined by comparison with

¹ Obtainable from Hynson, Westcott and Dunning.

² Rosenthal, S. M. An Improved Method for Using Phenoltetrachlorophthalein as a Liver Function Test. *J. Pharm. and Exp. Therap.* 10: 383-401 (June) 1922.

³ Keith, N. M., Rowntree, I. C. and Geraghty, J. T. A Method for the Determination of Plasma and Blood Volume. *Arch. Int. Med.* 16: 54-56 (Oct.) 1915.

⁴ Harris, D. T. The Value of the Vital red Method as a Clinical Means for the Estimation of the Volume of Blood. *Brit. Jour. Exper. Path.* 1: 142-158 1920.

See Graff, S. and Clarke, H. T. Determination of Plasma Volume I. The Dye Method. *Arch. Int. Med.* 48: 805-827 (Nov.) 1931 for a detailed consideration of this method.

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this standard Knowing the concentration of dye in the plasma and the amount of dye injected, the total plasma volume is readily calculated and knowing the total plasma volume and the plasma per cent of the whole blood the total blood volume may also be calculated The results are expressed in cc per kilogram of body weight

The formulae are $200 \times \text{number of cc of dye injected} \div \frac{\text{mm standard}}{\text{mm unknown}} = \text{plasma volume in cc}$

$$\frac{\text{Plasma volume in cc} \times 100}{\text{Plasma per cent}} = \text{whole blood volume in cc}$$

Since dry oxalate is used as an anticoagulant the plasma volume is approximately 3.5 per cent too high but if this technic is used in all cases the results will be comparable

(b) *For the Detection of Amyloidosis and Nephrosis*—Bennhold¹ discovered that congo red rapidly disappeared from the circulation in uncomplicated cases of nephrosis and amyloid disease

(1) *Principle* Congo red normally disappears slowly from the circulation but in amyloid disease it disappears rapidly due to an affinity for the amyloid substance In lipid nephrosis it is more rapidly excreted into the urine than in normals, and can be detected by its red color

(2) *Technic* Inject intravenously 10 to 15 cc or the amount calculated for use in determination of the total blood volume of 1.5 per cent aqueous solution of Grubler's congo red filtered and sterilized Four minutes later draw 10 cc of blood from a vein of the opposite arm and run it into a test tube containing 20 mg of powdered potassium oxalate and mix thoroughly at once At the end of 1 hour a second 10 cc sample of blood is taken in a similar manner Both are centrifugated The clear plasma is withdrawn and the colors compared in a colorimeter, using the four minute sample as the standard

$$\text{Calculation } \frac{\text{Mm four minute sample (e.g. 10)}}{\text{Mm one hour sample}} \times 100 = \text{per cent of dye still in plasma 100 minus the per cent of dye remaining in the plasma equals the per cent of dye which has disappeared}$$

Urine should be collected at the end of one hour, if the urine is red and turns blue on addition of hydrochloric acid and more than 40 per cent of the dye has disappeared from the blood stream, nephrosis is the most probable diagnosis

G *Miscellaneous Methods*—1 *Serum Bromides* (Wuth)—(a) *Principle* The serum proteins are precipitated with trichloroacetic acid The brownish gold bromide is formed and determined by comparison with standards in a biocolorimeter (Fig 9)

(b) *Technic*—To 2 cc of serum add 4 cc of water and 1.2 cc of 20 per cent trichloroacetic acid, and filter or centrifugate To 2 cc of filtrate add 0.4 cc of 0.5 per cent gold chloride mix, and match with the standards interpolating if necessary

¹ Bennhold H Über die Beziehungen des Kongorotes zur amyloiden Substanz und über den Mechanismus der Beschleunigten Farbstoffausscheidung bei Tubulären Nierenkrankheiten Klin Wchnschr 3 part 1 1711 1712 (September 16) 1924

(c) *Preparation of Standards*¹—Dissolve 193 mg of anhydrous sodium bromide in 50 cc of water and add the reagents in the order given in the table to test tubes of uniform diameter, labelled as in the first column. Cork, seal with paraffin, and keep in the dark when not in use.

Value in Mg	Sodium Bromide cc	Water cc	Trichloroacetic Acid 20 Per Cent cc	Gold Chloride Sol 0.5 Per Cent cc
300	2.0	4.0	1.2	1.4
200	1.3	4.67	1.2	1.4
150	1.0	5.0	1.2	1.4
125	0.85	5.15	1.2	1.4
100	0.67	5.33	1.2	1.4
75	0.50	5.50	1.2	1.4
0	0.00	6.0	1.2	1.4

2 *Tests for Carbon Monoxide Hemoglobin*²—Many methods for detecting carbon monoxide hemoglobin have been devised but, in my experience, the simple methods are too inaccurate and the accurate methods are too complicated for clinical use. The following method has proved satisfactory. The blood for examination should be taken within 3 hours after cessation of exposure to the supposed source of poisoning since 50 to 30 per cent of the carbon monoxide in combination with hemoglobin at the beginning of any hour is freed by the end of the hour. If the patient survives carbon monoxide hemoglobin will have disappeared from the blood within less than 24 hours, even after the heaviest exposures.

(a) *Technic*—Perform a hemoglobin estimation by the Osgood Haslins method, or some other reliable acid hematin method, on the patient's blood and on blood from a person known not to have been exposed to carbon monoxide within the last 24 hours. Dilute 1 cc of the patient's blood to 100 cc with distilled water and dilute the calculated amount of the normal blood to 100 cc to give a final dilution containing the same amount of hemoglobin. For example, if the patient's blood contains 12 gm of hemoglobin per 100 cc and the normal blood contains 16 gm of hemoglobin per 100 cc, dilute 0.75 cc of the normal blood to 100 cc. Mix thoroughly and compare in a colorimeter. If the colors are identical and the readings the same, no significant amount of carbon monoxide is present in the patient's blood. If the colors fail to match and the patient's blood appears more purplish red than the normal and the nearest match in intensity is obtained

¹ Reagents and standards ready prepared may be obtained from the La Motte Chemical Products Co. or from the Shaw Surgical Co. Portland, Ore.

² Osgood, E. E. and Ashworth, Clarice M. *Atlas of Hematology*. Pp. 207-208. J. W. Stacey, Inc. San Francisco, 1937.

with a lower colorimeter reading in the unknown (i.e. normal at 15, patient's at 12.8 mm), carbon monoxide hemoglobin is present. The amount may be determined by dividing the diluted known normal blood into two portions. Completely saturate one by bubbling illuminating gas or pure carbon monoxide through it under a hood and make a series of mixtures of this 100 per cent carbon monoxide hemoglobin and the 100 per cent oxyhemoglobin to cover a range of percentages at about 10 per cent intervals from 10 to 50 per cent (e.g. 2 cc carbon monoxide hemoglobin + 18 cc oxyhemoglobin = 10 per cent, 4 cc carbon monoxide hemoglobin + 16 cc oxyhemoglobin = 20 per cent, etc). Determine in the colorimeter which of these mixtures most nearly matches the unknown in color and intensity and thus will give the percentage of carbon monoxide hemoglobin present. No symptoms are ordinarily present unless the concentration is above 20 per cent and concentrations above 50 per cent occur only in patients who are in coma.

3 Test for Detection of Methemoglobin and Sulphemoglobin¹ in Blood—A technic similar to that given for carbon monoxide hemoglobin is suitable for the detection of methemoglobin. Proceed as described above to the stage of the first comparison in the colorimeter. If methemoglobin is present the unknown will be a much deeper brownish red than the normal when the cups are set at the same depth, and the nearest match in intensity will be with a much lower reading for the unknown than for the oxyhemoglobin. To determine the quantity of methemoglobin present, a colorimeter having two cups and plungers on one side of the type used for pH determinations must be used. Put the dilution of the unknown on the side of the single plunger and add sodium nitrite pills or crystals to one half of the oxyhemoglobin, changing it all to methemoglobin. Place part of this in the lower cup and part of the oxyhemoglobin in the upper cup of the colorimeter and adjust the ratchets until the colors match in both intensity and character. Read the verniers and determine the percentage of methemoglobin by the ratio of the vernier reading for the plunger dipped in the 100 per cent methemoglobin to the sum of this reading plus the oxyhemoglobin reading which should equal the reading of the unknown. For example, if the unknown is set at 15 mm, the oxyhemoglobin reads 10 mm, and the methemoglobin reads 5 mm, the blood contains 33 per cent of its hemoglobin in the form of methemoglobin and the actual amount of methemoglobin is 33 per cent of the grams of hemoglobin per 100 cc as determined by the acid hematin method.

¹ Healy J. C. Sulphemoglobinemia. *J. Lab. and Clin. Med.* 18: 348 (Jan.) 1933.

If the brown color is due to methemoglobin, addition of 1 or 2 drops of ammonium sulphide and shaking vigorously will restore the oxyhemoglobin color, whereas, if the brown color is due to sulphemoglobin, no change will occur

4 **Quantitative Determination of Sulfanilamide and Sulfapyridine** (Marshall¹ as modified by Marshall and Litchfield¹)—The quantities given are for determination of free sulfanilamide or sulfapyridine alone. If the conjugated form is to be determined too, double the quantities

(a) *Principle*—The NH_2 group attached to the benzene ring in sulfanilamide or any of its derivatives in which the NH_2 group is free is diazotized by the action of nitrous acid and coupled with dimethyl- α naphthylamine or N (1 naphthyl) ethylenediamine² to form a pink dye. The intensity of the color produced is compared colorimetrically with a standard of known sulfanilamide or sulfapyridine content

(b) *Technic*—Into a flask, measure 20 cc of oxalated blood and add 14 cc of 0.05 per cent *saponin solution*. Let this stand two minutes or more and add 40 cc of 15 per cent *trichloroacetic acid solution*. Distilled water may be used instead of saponin but laking then requires 10 minutes or more and should be complete before addition of the trichloroacetic acid. Mix thoroughly, let stand 5 minutes or more, and filter. To 10 cc of the filtrate and to 10 cc of dilute standard sulfanilamide or sulfapyridine in another tube, add 10 cc of freshly prepared 0.1 per cent sodium nitrite. Mix, let stand 3 minutes, and to each add 1 cc of buffered 0.5 per cent *ammonium sulfamate*. Let this stand 2 minutes and add 50 cc of alcoholic solution of dimethyl α naphthylamine. Let this stand 10 minutes and compare in a colorimeter. If the acetyl sulfanilamide or acetyl sulfapyridine is to be determined, prepare a double volume of filtrate as above and treat 10 cc as described above. To another 10 cc add 20 cc of *N/1 hydrochloric acid* and heat in a boiling water bath for one hour. Cool and dilute to 10 cc. Then add reagents as to the filtrate except that the buffered sulfamate solution should contain 2M instead of 1M sodium acid phosphate

(c) *Calculation*—
$$\frac{\text{The colorimeter reading of standard}}{\text{The colorimeter reading of unknown}} \times \text{factor} =$$

mg of sulfanilamide or sulfapyridine per 100 cc of blood. The factor which should be used in the calculation is 10, 5 or 2, depending on which

¹ Marshall I. K. Jr. Determination of Sulfanilamide in the Blood and Urine. J Biol Chem 122: 263-273 (Dec) 1937.

² Marshall I. K. Jr and Litchfield J. T. Jr. The Determination of Sulfanilamide. Science 83: 85-86 (July 22) 1938.

³ Bratton A. C. and Marshall E. K. Jr. A New Coupling Component for Sulfanilamide Determination. J Biol Chem 118: 537-550 (May) 1939.

standard is used since they are matched against a 1-10 dilution of the blood Total — free = sulfanilamide or sulfapyridine combined with acetyl radicle

(d) *Reagents* —Saponin solution Dissolve 0.5 grams of saponin in 1 liter of distilled water

Buffered 0.5 per cent ammonium sulfamate Dissolve 13.8 grams of NaH_2PO_4 , H_2O and 0.5 grams of ammonium sulfamate (LaMott Chemical Products Co., Baltimore) in distilled water and dilute to 100 cc. Use 27.6 grams of NaH_2PO_4 , H_2O and 0.5 grams of ammonium sulfamate per 100 cc. to make the 2M buffered sulfamate solution for the determination of total sulfanilamide or sulfapyridine

Dimethyl- α -naphthylamine Dissolve 1 cc. in 250 cc. of 95 per cent ethyl alcohol. A blank determination with this solution when used with 10 cc. of distilled water instead of filtrate should give no pink color and only a slight yellow color in 10 minutes, with the standard sulfanilamide solution it should give a pink color which does not increase in intensity after 10 minutes. If color development is slow the dimethyl- α -naphthylamine should be heated on an oil bath at 265° in a test tube while a stream of air is bubbled through it through capillary glass tubing

Standard sulfanilamide or sulfapyridine Dissolve 200 mg. of the drug in 800 cc. of hot distilled water, cool, and dilute to 1 liter. Prepare the dilute standards from these by measuring into volumetric flasks, 5, 2.5 and 1 cc. of the stock solution, adding 18 cc. of 15 per cent trichloroacetic acid and diluting to 100 cc. with distilled water. These standards contain respectively 10, 0.5 and 0.2 mg. per 100 cc. The factor used in calculation is 10 times these figures

5 **Cevitamic Acid Determination** (Farmer and Abt¹) —Both a macrodetermination and a microdetermination are available. The macrodetermination is recommended if blood is obtainable by venipuncture or from the fontanel. The microdetermination is recommended for children with small veins who are too old for fontanel puncture

(a) *Principle* —The dye, 2,6-dichlorophenolindophenol is reduced to a colorless form by the reduced cevitamic acid in a protein free filtrate from the blood

(b) *Technic of the Macrodetermination* —Centrifuge 5 cc. of oxalated venous blood as soon as possible after withdrawal. To 2 cc. of the plasma in a centrifuge tube add 4 cc. of distilled water and 2 cc. of freshly prepared 5 per cent metaphosphoric acid. Mix thoroughly and centrifuge. Transfer 2 cc. of the protein free supernatant fluid to another centrifuge tube and titrate with the dye solution, using a 5 cc. microburette, to the faintest detectable pink color which persists for 20 to 30 seconds after mixing

¹Farmer C. J. and Abt A. F. Ascorbic Acid Content of Blood. Proc. Soc. Exper. Biol. & Med. 32: 1625-1629 (June) 1935

Farmer C. J. and Abt A. F. Determination of Reduced Ascorbic Acid in Small Amounts of Blood. Ibid. 34: 146-150 (Mar) 1936

(1) Calculation—Cc of dye $\times 4^* =$ mg of reduced cevitic acid per 100 cc of plasma

(c) *Technic of Microdetermination*—Make a deep puncture with a lancet in the heel or toe and collect 6 to 8 drops of blood in a small phial¹ containing 2 mg of potassium oxalate. Cork and shake thoroughly. Place the phial in a recessed cork in a centrifuge tube and centrifuge. With the 0.1 cc pipette, transfer 0.1 cc of plasma to a 15 cc centrifuge tube and with the same pipette add 0.1 cc of distilled water. With a second micropipette, transfer 0.2 cc of freshly prepared 5 per cent metaphosphoric acid to the same tube. Mix thoroughly by tapping and centrifuge. With a micropipette, transfer 0.2 cc of the deproteinized fluid to a depression in the porcelain tile. Into a neighboring depression, pipette 0.1 cc of 5 per cent metaphosphoric acid and 0.1 cc of distilled water. Fill the bulb of the microburette partly full of mercury and turn the screw clamp until a drop of mercury appears at the tip. Place the tip below the surface of a few cubic centimeters of the standard dye solution in a tilted test tube and turn the screw clamp until a drop of mercury is extruded into the dye. Reverse the direction of turning until the microburette is filled with the dye solution. Slip off the test tube containing the dye and slide the tile under the tip and titrate the fluid in both depressions to the faintest pink color which persists for 30 seconds. Comparison may be made with the color of 0.2 cc of distilled water in another depression as an aid in determining the end point. Read the microburette which is calibrated in 0.002 cc divisions.

(1) Calculation—Cc of dye—cc used in the control $\times 40^\dagger =$ mg of cevitic acid per 100 cc of plasma

(2) Reagents—Place one tablet of sodium 2,6-dichlorophenolindophenol² in a 50 cc volumetric flask and dilute to the mark with distilled water. This should be checked from time to time against a standard cevitic acid solution made up by dissolving the contents of a 0.1 gm vacuum filled ampule (Merck cebione) in 5 per cent acetic acid made from freshly boiled and cooled distilled water in a 100 cc volumetric flask and dilute to the mark. To 2 cc of this solution in a 50 cc volumetric flask add enough 5 per cent acetic acid to fill to the mark. Mix and titrate 2 cc of this solution in a centrifuge tube. The volume of dye solution required should be 2 cc. A factor may be calculated to substitute for the 0.02 in the formula if the titration value of the dye solution is changed

* 1 cc of dye = 0.02 mg and 0.5 cc of plasma = $\frac{1}{200}$ of 100 cc $0.02 \times 200 = 4$

¹ The phial, porcelain plate, microburette and micropipettes are obtainable from E. H. Sargent & Co. Chicago.

[†] 1 cc of dye solution = 0.02 mg of cevitic acid. 0.2 cc of protein free fluid = 0.05 cc of plasma which is $\frac{1}{2000}$ of 100 cc $2000 \times 0.02 = 40$

² Obtainable from E. H. Sargent & Co. Chicago

I Quantity of Blood Needed for Each Method

TABLE 23

	Blood	Filtrate
Blood urea		
Aeration method	5 cc	
Urea clearance	5 cc	5 cc
Non protein nitrogen	2 cc	10 cc
Blood creatinine	2 cc	10 cc
Blood uric acid	2 cc	5 cc.
Blood dextrose	2 cc	5 cc
Blood chlorides	2 cc	10 cc
Alkali reserve	5 cc	2 c of plasma
Blood cholesterol	2 cc	
Blood phosphorus	5 cc	2 cc plasma or serum.
Blood calcium	5 cc *	2 cc serum
Icterus index	5 cc *	2 cc serum or plasma
Van den Bergh	3 cc *	1 cc. of serum or plasma
Folin's complete analysis	7 cc	50 cc
Hematologic examination [†]	5 cc	
Blood proteins	5 cc *	1 or 2 cc serum.
	5 cc	1 or 2 cc plasma
Blood bromide	5 cc *	2 cc serum.

* No anticoagulant

[†] See the uniform system of hematologic methods (p. 461)

Note These are the amounts to be measured, hence add at least 0.5 cc, preferably over 1 cc, to the sum of the amounts given for the estimations desired. *It is still better to take 5 cc more than the amounts specified as this will permit a recheck of any determination should question as to its accuracy arise.* Oxalated blood is satisfactory for all methods, except the blood calcium and globulin determinations.

SECTION V BASAL METABOLIC RATE DETERMINATION¹

For a discussion of energy metabolism, the factors, both physiologic and pathologic, which influence the basal metabolic rate, and the general instructions to the patient and technician necessary for its accurate determination, see page 103. It is important that the technician be calm and unhurried in her directions and in the performance of the test since an atmosphere of tranquillity is absolutely essential.

On the morning of the test assure yourself (a) that your instructions have been followed, (b) that no contraindications to doing the test have developed since you last saw the patient, (c) that the patient has had a good night's sleep, (d) that the patient has urinated before starting the rest period.

A The Rest Period—See that the room temperature is comfortable and that there are no drafts. The room must be quiet. The patient is to lie on his back on a comfortable couch with the head supported by a thin pillow. The clothing is to be loosened sufficiently for comfort. Darken the room or lay a folded cloth over the eyes to protect them from the light. Tell the patient to relax physically and mentally as much as possible just as if he were trying to go to sleep. The rest period has been shown to decrease the basal metabolic rate from 8 to 10 per cent. At least 30 minutes is desirable for this period. In a few cases the patient is more comfortable in a semi reclining position and a more accurate result is secured.

B Technic of the Test²—Note the patient's pulse at intervals during the test and record any evidence of nervousness or tension. To prepare the apparatus for the test, attach the breathing tubes, cork the end of the tubes, see that the respiratory valves are working, insert the box of soda lime, to about 2 liters of air in the float add 2 liters of oxygen or 3 if a high rate is expected and see that the pen and recording apparatus are in order. After the 30 minute rest period attach the clean sterilized rubber mouthpiece and insert it in the

¹ Roth P. and Buckingham Pearl E. The Criteria of a Dependable Basal Metabolism Report. Am J Clin Path 9: 79-92 (Jan) 1930.

² The directions given are for use of the McKesson metabor, but only slight changes are necessary for any of the machines for determining the metabolism by the measurement of oxygen consumption alone. The Tissot spirometer method is too difficult for clinical use in any but the largest laboratories although it is the most accurate for research.

TABLE 25—SURFACE AREA IN

Weight in pounds	Height in inches																			
	40	41	42	43	44	45	46	47	48	49	50	51	52	53	54	55	56	57	58	
40	70	71	72	74	75	76	77	78	80	81	82	83	84	86	87	88	89	90	91	
45	74	75	76	78	79	80	81	82	84	85	86	87	88	90	91	92	93	94	96	
50	77	78	79	81	82	83	85	86	87	88	90	91	92	94	95	96	98	99	100	
55	80	82	83	84	85	87	89	90	91	92	94	95	96	98	99	100	101	102	104	
60	83	85	86	87	88	90	92	93	94	95	97	99	100	101	102	104	105	107	108	
65	86	88	89	90	91	93	94	96	98	100	101	103	104	105	106	108	109	111	112	
70	88	90	91	93	94	96	97	99	101	103	104	106	107	108	109	111	113	114	115	
75	91	93	94	96	97	99	100	102	104	106	107	109	110	112	113	115	117	118	119	
80	93	95	97	98	100	102	103	105	107	109	110	112	113	115	116	118	120	121	122	
85	96	98	100	101	103	105	106	108	110	112	113	115	116	118	119	121	123	124	125	
90	98	100	102	103	105	107	108	110	112	114	115	117	118	120	121	123	125	127	128	
95	101	103	105	106	108	110	111	113	115	117	118	120	121	123	125	127	129	130	132	
100	103	105	107	108	110	112	114	116	118	120	121	123	124	126	128	130	131	133	134	
105	105	107	109	110	112	114	116	118	120	122	124	126	127	129	131	133	134	136	137	
110	107	109	111	112	114	116	118	120	122	124	126	128	130	131	133	135	136	138	140	
115	109	111	113	115	117	119	121	123	125	127	129	131	133	134	136	138	139	141	143	
120	111	113	115	117	119	121	123	125	127	129	131	133	135	136	138	140	142	144	145	
125	113	115	117	119	121	123	125	127	130	132	134	136	138	139	141	143	145	147	148	
130	115	117	119	121	123	125	127	129	132	134	136	138	140	141	143	145	147	149	150	
135	117	119	121	123	125	127	129	131	134	136	138	140	142	143	145	147	149	151	153	
140	118	121	123	125	127	129	131	133	135	137	139	141	143	145	147	149	151	153	155	
145	120	123	125	127	129	131	133	135	137	139	141	143	145	148	150	152	154	156	158	
150	122	125	127	129	131	133	135	137	139	141	143	145	147	150	152	154	156	158	160	
155	123	125	127	130	132	135	137	139	141	143	145	147	149	152	154	156	158	160	162	
160	125	127	129	132	134	137	139	141	143	145	147	149	151	154	156	158	160	162	164	
165	127	129	131	134	136	139	141	143	145	147	149	151	153	156	158	160	162	164	167	
170	129	131	133	136	138	140	142	144	147	149	151	153	155	158	160	162	164	166	169	
175	131	133	135	138	140	142	144	146	149	151	153	155	157	160	162	164	166	168	171	
180	132	134	136	139	141	143	145	148	150	153	155	157	159	162	164	166	168	170	173	
185	134	136	138	141	143	145	147	150	152	155	157	159	161	164	166	168	170	172	175	
190	135	137	140	142	145	147	149	152	154	157	159	161	163	166	169	171	173	174	177	
195	137	139	142	144	147	149	151	154	156	159	161	163	165	168	170	172	174	176	179	
200	138	140	143	145	148	150	152	155	157	160	162	165	167	169	171	174	176	178	181	
205	140	142	145	147	150	152	154	157	159	162	164	167	169	171	173	176	178	180	183	
210	141	143	146	148	151	153	155	158	160	163	165	168	170	173	175	178	180	182	185	
215	143	145	148	150	153	155	157	160	162	165	167	170	172	175	177	180	182	184	187	
220	144	146	149	151	154	156	159	161	164	166	169	172	174	177	179	181	184	186	188	
225	145	148	150	153	155	158	161	163	166	168	171	174	176	178	180	183	185	188	190	
230	146	149	151	154	156	159	162	164	167	169	172	175	177	180	181	184	187	189	191	
235	148	151	153	156	158	161	164	166	169	171	174	177	179	181	183	186	189	191	193	
240	149	152	154	157	159	162	165	167	170	172	175	178	180	182	184	187	190	192	195	
245	151	154	156	159	161	164	167	169	172	174	177	179	181	184	186	189	192	194	197	
250	152	155	157	160	162	165	168	170	173	175	178	180	182	185	187	190	193	195	198	
255	153	156	158	161	163	166	169	171	174	176	179	181	183	186	188	191	194	197	200	
260	154	157	159	162	164	167	170	173	175	178	181	183	185	188	190	193	196	198	201	
265	156	159	161	164	166	169	171	174	177	180	183	185	187	190	192	195	198	200	203	
270	157	160	162	165	167	170	173	176	179	181	184	187	189	192	194	197	200	202	205	
275	158	161	164	166	169	172	175	178	181	183	186	189	191	194	196	199	202	204	207	
280	159	162	165	167	170	173	176	179	182	184	187	190	192	195	197	200	203	205	208	
285	160	163	166	168	171	174	177	180	183	186	189	192	194	197	199	202	205	207	210	
290	161	164	167	169	172	175	178	181	184	187	190	193	195	198	200	203	206	208	211	
295	162	165	168	170	173	176	179	182	185	188	191	194	196	199	202	204	207	209	212	

* From formula of Dubois & Dubois $A = W^{.725} \times H^{.725}$

SQUARE METERS*

Height in inches

59	60	61	62	63	64	65	66	67	68	69	70	71	72	73	74	75	76	77
92	93	94	95	97	98	99	100	101	102	103	104	106	107	108	109	110	111	112
97	98	99	100	102	103	104	106	107	108	109	110	112	113	114	115	116	117	118
101	103	104	105	107	108	109	111	112	113	114	115	117	118	119	120	121	122	123
105	107	108	109	111	112	113	115	116	117	118	119	120	122	123	124	125	126	128
109	111	112	113	115	116	117	119	120	121	122	124	126	127	128	129	130	131	132
113	115	117	118	119	120	121	122	124	125	126	127	128	130	131	132	133	135	137
116	118	119	121	123	125	126	128	129	130	131	132	134	135	136	137	139	140	141
120	122	123	125	127	129	130	132	133	134	135	136	138	139	140	141	143	144	145
123	125	126	128	130	132	133	135	136	137	138	140	142	143	144	145	147	148	149
126	128	130	132	133	135	137	139	140	141	142	144	146	147	148	149	151	152	153
129	131	133	135	136	138	140	142	143	144	145	147	149	150	152	153	155	156	157
133	135	136	138	140	142	143	145	146	148	149	151	153	154	156	157	159	160	161
136	138	139	141	143	145	146	147	149	151	153	154	155	157	159	161	162	163	165
139	141	142	144	146	148	149	150	152	154	156	157	159	161	162	164	166	167	169
142	143	145	147	148	150	152	153	155	157	159	160	162	164	165	167	169	170	172
145	146	148	150	151	153	155	156	158	160	162	163	165	167	168	170	172	173	175
147	149	151	153	154	156	158	159	161	163	165	166	168	170	171	173	175	176	178
150	152	154	156	157	159	161	162	164	166	168	169	171	173	174	176	178	179	181
152	154	156	158	159	161	163	165	167	168	170	172	174	176	177	179	181	182	184
155	157	159	161	162	164	166	168	170	171	173	175	177	179	180	182	184	185	187
157	159	161	163	164	166	168	170	172	174	176	178	180	182	183	185	187	188	190
160	162	164	166	167	169	171	173	175	177	179	181	183	185	186	188	190	191	193
162	164	166	168	169	171	173	175	177	179	181	183	185	187	189	191	193	195	197
164	166	168	170	172	174	176	178	180	182	184	186	188	190	192	194	196	198	200
166	168	170	172	174	176	178	180	182	184	186	188	190	192	194	196	198	200	202
169	171	173	175	177	179	181	183	185	187	189	191	193	195	197	199	201	203	205
171	173	175	177	179	181	183	185	187	189	191	193	195	197	199	201	203	205	207
173	175	177	179	181	183	185	187	189	191	193	195	197	199	201	203	205	207	209
175	177	179	181	183	185	187	189	191	193	195	197	199	201	203	205	207	209	211
177	179	181	183	185	187	189	191	193	195	197	199	201	203	205	207	209	211	213
179	181	183	185	187	189	191	193	195	197	199	201	203	205	207	209	211	213	215
181	183	185	187	189	191	193	195	197	199	201	203	205	207	209	211	213	215	217
183	185	187	189	191	193	195	197	199	201	203	205	207	209	211	213	215	217	219
185	187	189	191	193	195	197	199	201	203	205	207	209	211	213	215	217	219	221
187	189	191	193	195	197	199	201	203	205	207	209	211	213	215	217	219	221	223
189	191	193	195	197	199	201	203	205	207	209	211	213	215	217	219	221	223	225
190	193	195	197	199	201	203	205	207	209	211	213	215	217	219	221	223	225	227
192	195	197	199	201	203	205	207	209	211	213	215	217	219	221	223	225	227	229
193	196	199	201	203	205	207	209	211	213	215	217	219	221	223	225	227	229	231
195	198	201	203	205	207	209	211	213	215	217	219	221	223	225	227	229	231	233
197	200	203	205	207	209	211	213	215	217	219	221	223	225	227	229	231	233	235
199	202	205	207	209	211	213	215	217	219	221	223	225	227	229	231	233	235	237
200	203	206	208	211	213	215	217	219	221	223	225	227	229	231	233	235	237	239
202	205	208	210	213	215	217	219	221	223	225	227	229	231	233	235	237	239	241
203	206	209	211	213	215	217	219	221	223	225	227	229	231	233	235	237	239	241
205	208	211	213	215	217	219	221	223	225	227	229	231	233	235	237	239	241	243
207	210	213	215	217	219	221	223	225	227	229	231	233	235	237	239	241	243	245
209	213	215	217	219	221	223	225	227	229	231	233	235	237	239	241	243	245	247
210	213	216	218	220	222	225	227	229	231	233	235	237	239	241	243	245	247	249
212	215	218	220	222	224	227	229	231	233	235	237	239	241	243	245	247	249	251
213	216	219	221	223	225	227	229	231	233	235	237	239	241	243	245	247	249	251
214	217	220	222	224	226	228	231	233	235	237	239	241	243	245	247	249	251	253
217	220	223	225	227	229	231	233	235	237	239	241	243	245	247	249	251	253	255

plete instructions for handling each machine are furnished by the manufacturer

C Calculation—Lay the record on a table, apply the edge of a ruler to the lowest points of the curves, if most of these points practically coincide with a straight line and there are no marked divergences, then the slope of the curves is uniform, draw a line and use the entire record for calculation. If the tracing is not regular, find a portion where the slope of the curves is uniform for an interval of several minutes and draw the line

TABLE 26—BASAL METABOLIC RATE NORMALS (THE SAGE STANDARDS)

Ages	Cal per hr per sq m		Ages	Cal per hr per sq m	
	Males	Females		Males	Females
14-16	46 0	43 0	40-50	38 5	36 0
16-18	43 0	40 0	50-60	37 5	35 0
18-20	41 0	38 0	60-70	36 5	34 0
20-30	39 5	37 0	70-80	35 5	33 0
30-40	39 5	36 5			

Read the volume of oxygen used in a period of 3 to 5 minutes from the factors for the graph paper used in the machine employed. With the Metabolor, each small square equals 100 cc of oxygen or 0.1 minute. Divide the cc of oxygen by the minutes to obtain the cc of oxygen consumed per minute. In some machines the graph paper is so calibrated that corrections are automatically made for barometric pressure and temperature. These are satisfactory for clinical purposes but are not so satisfactory for teaching students the principles of the method as is a machine such as the Metabolor which makes readings in actual cubic centimeters. Multiply the result¹ by the factor in Table 24 that corresponds to the barometric pressure and the average temperature. This gives the total calories per hour.² From Table 25,³ determine the surface area of the patient in square meters, where height and weight intersect. The basal metabolic rate in calories per hour per square meter is secured by dividing the total calories by the square meters of area or by referring to Table (C) which comes with the apparatus.

¹ If using the charts furnished with the Metabolor multiply by 0.06 to convert to liters per hour and then multiply by the factor found in Chart A.

² To use the Harris and Benedict standards continue as directed on p. 429 if a child continue as directed on p. 432.

³ Reproduced by permission of the McKesson Appliance Co.

NORMALS (THE HARRIS BENEDICT STANDARDS)¹

Directions The predicted calories per hour are obtained by adding the calories corresponding to the weight in kilograms (Table 27) to the calories corresponding to age and stature (Table 28 29 or 30) The patient's metabolic rate is then calculated as per cent above or below the normal by multiplying by 100 the observed total calories per hour (not calories per square meter per hour) dividing by the predicted calories per hour, and subtracting 100 (see section C above)

In the use of these condensed tables ² interpolation is necessary

TABLE 27—HARRIS-BENEDICT STANDARDS BASED ON BODY WEIGHT

Weight, kilograms	Calories per hour		Weight, kilograms	Calories per hour	
	Males	Females		Males	Females
10	8 5		72	44 0	56 0
12	9 7		74	45 2	56 8
14	10 8		76	46 3	57 6
16	12 0		78	47 5	58 4
18	13 1		80	48 6	59 2
20	14 3		82	49 7	60 0
22	15 4		84	50 9	60 8
24	16 6		86	52 0	61 6
26	17 7	37 6	88	53 2	62 4
28	18 8	38 4	90	54 3	63 2
30	19 9	39 2	92	55 5	64 0
32	21 1	40 0	94	56 6	64 8
34	22 2	40 8	96	57 8	65 6
36	23 4	41 6	98	58 9	66 4
38	24 5	42 4	100	60 1	67 2
40	25 7	43 2	102	61 2	68 0
42	26 8	44 0	104	62 4	68 8
44	28 0	44 8	106	63 5	69 6
46	29 1	45 6	108	64 7	70 4
48	30 3	46 4	110	65 8	71 2
50	31 4	47 2	112	67 0	72 0
52	32 6	48 0	114	68 1	72 8
54	33 7	48 8	116	69 3	73 6
56	34 9	49 6	118	70 4	74 4
58	36 0	50 4	120	71 0	75 2
60	37 2	51 2	122	72 7	76 0
62	38 3	52 0	124	73 9	76 8
64	39 5	52 8	126	75 0	77 6
66	40 6	53 6	128	76 1	78 4
68	41 8	54 4	130	77 2	79 2
70	42 9	55 2			

¹ Harris J A and Benedict P G A Biometric Study of Basal Metabolism in Man Carnegie Inst of Wash., Pub 270 Pp 272 1919

Carpenter T M Tables Factors and Formulas for Computing Respiratory Exchange and Biological Transformations of Energy Carnegie Inst. of Wash. Pub 303 Pp 125 1924

² Reproduced by permission from the Metabolometric Chart calculated and condensed by Paul Roth from the original papers. Less condensed tables will be found in his Metabolometric Compendium which is obtainable from W F Collier 355 Huntington Ave Boston Mass.

TABLE 28—HARRIS BENEDICT STANDARDS BASED ON AGE AND STATURE—MEN

Cm	Age										
	20	25	30	35	40	45	50	55	60	65	70
150	25 6	24 2	22 8	21 4	20 0	18 6	17 2	15 8	14 4	13 0	11 6
155	26 6	25 2	23 8	22 4	21 0	19 6	18 2	16 8	15 4	14 0	12 6
160	27 7	26 3	24 9	23 5	22 1	20 7	19 3	17 9	16 5	15 1	13 7
165	28 7	27 3	25 9	24 5	23 1	21 7	20 3	18 9	17 5	16 1	14 1
170	29 8	28 4	27 0	25 6	24 2	22 8	21 4	20 0	18 6	17 2	15 8
175	30 8	29 4	28 0	26 6	25 2	23 8	22 4	21 0	19 6	18 2	16 8
180	31 9	30 4	29 1	27 6	26 2	24 8	23 4	22 0	20 6	19 2	17 8
185	32 9	31 5	30 1	28 7	27 3	25 9	24 5	23 1	21 7	20 3	18 9
190	34 0	32 5	31 2	29 7	28 3	26 9	25 5	24 1	22 7	21 3	19 9
195	35 0	33 6	32 2	30 8	29 4	28 0	26 6	25 2	23 8	22 4	21 0
200	36 1	34 6	33 2	31 8	30 4	29 0	27 6	26 2	24 8	23 4	22 0

TABLE 29—HARRIS BENEDICT STANDARDS BASED ON AGE AND STATURE—WOMEN

150	7 7	6 7	5 7	4 7	3 8	2 8	1 8	0 9	0 0	-1 0	-2 0
155	8 1	7 1	6 1	5 1	4 2	3 2	2 2	1 2	0 2	-0 7	-1 7
160	8 5	7 5	6 5	5 5	4 5	3 6	2 6	1 6	0 6	-0 3	-1 3
165	8 8	7 8	6 9	5 9	4 9	4 0	3 0	2 0	1 0	0 0	-0 9
170	9 2	8 2	7 3	6 3	5 3	4 3	3 4	2 4	1 4	0 5	-0 5
175	9 6	8 6	7 6	6 7	5 7	4 7	3 7	2 8	1 8	0 8	-0 2
180	10 0	9 0	8 0	7 0	6 1	5 1	4 1	3 2	2 2	1 2	0 2
185	10 4	9 4	8 4	7 5	6 5	5 5	4 5	3 5	2 6	1 6	0 6
190	10 8	9 8	8 8	7 8	6 8	5 9	4 9	3 9	3 0	2 0	1 0
195	11 2	10 2	9 2	8 2	7 2	6 2	5 3	4 3	3 3	2 4	1 4
200	11 5	10 5	9 6	8 6	7 6	6 7	5 7	4 7	3 7	2 7	1 8

TABLE 30—HARRIS BENEDICT STANDARDS BASED ON AGE AND STATURE—BOYS

Cm	Age			Cm	Age		
	10	15	20		10	15	20
100	18 0	16 6	15 2	155	29 5	28 1	26 6
105	19 0	17 7	16 3	160	30 5	29 1	27 7
110	20 0	18 7	17 3	165	31 5	30 1	28 7
115	21 0	19 7	18 3	170	32 6	31 2	29 8
120	22 1	20 8	19 4	175	33 6	32 2	30 8
125	23 2	21 8	20 4	180	34 7	33 3	31 9
130	24 2	22 9	21 5	185	35 7	34 3	32 9
135	25 3	23 9	22 5	190	36 8	35 4	34 0
140	26 3	25 0	23 6	195	37 8	36 4	35 0
145	27 4	26 0	24 6	200	38 9	37 4	36 1
150	28 4	27 0	25 6				

For clinical purposes this is changed to per cent of the average normal. Multiply the calories per sq meter per hour by 100 and divide by the normal for the same sex and age (Table 26). If the result is greater than 100, there is a plus rate, if less than 100, a minus rate. Take the difference between the figure and 100, then prefix plus or minus, for example, suppose the basal metabolic rate is 85 per cent of the normal, then the rate is minus 15 or 15 per cent below normal, and if the basal metabolic rate is 120 per cent of the normal, the rate is plus 20 or 20 per cent above normal. Calculate the rate from the formula given on page 111 from the pulse rate and pulse pressure and if there is a great discrepancy have the patient return for a recheck. The first metabolic rate determination is often too high, hence it is a

TABLE 31—STANDARD TOTAL CALORIES FOR WEIGHT—GIRLS AND BOYS¹

Weight kg	Total calories per hour		Weight kg	Total calories per hour	
	Girls	Boys		Girls	Boys
3 0	5 7	6 3	38 0	50 3	54 4
4 0	8 5	8 8	40 0	51 7	55 8
5 0	11 4	11 3	42 0	53 1	57 1
6 0	14 0	13 8	44 0	54 4	58 3
7 0	16 5	16 3	46 0	55 8	59 6
8 0	18 7	18 5	48 0	57 1	60 8
9 0	20 7	20 6	50 0	58 3	61 9
10 0	22 5	22 7	52 0	59 5	62 7
11 0	24 3	24 8	54 0	60 8	64 8
12 0	25 8	26 0	56 0	62 1	65 8
13 0	27 3	27 7	58 0	63 2	66 7
14 0	28 6	29 2	60 0	64 3	67 9
15 0	29 9	30 2	62 0	65 5	69 2
16 0	31 1	31 3	64 0	66 6	70 4
17 0	32 3	32 5	66 0	67 8	71 8
18 0	33 4	33 8	68 0	68 9	73 5
19 0	34 5	35 0	70 0	70 0	74 4
20 0	35 5	36 3	72 0	71 0	75 6
22 0	37 4	37 9	74 0	72 1	76 9
24 0	39 3	40 8	76 0	73 2	77 9
26 0	41 0	44 6	78 0	74 2	79 2
28 0	42 7	45 8	80 0	75 2	
30 0	44 7	47 5	82 0	76 3	
32 0	45 9	49 6	84 0	77 4	83 3
34 0	47 4	51 3			
36 0	48 9	52 9			

¹ Reproduced by permission of the author and publisher from Talbot F B Basal Metabolism Standards for Children. Am. J. Dis. Child. 55: 455-459 (March) 1938.

The standards are calculated in calories per hour instead of calories per 24 hours as in the original.

good rule to recheck all high rates and it is safer to do at least two tests on different days on all patients

For children, calculate the total calories per hour and express the results in percentage of normals for weight or height in Tables 31 or 32

TABLE 32—STANDARD TOTAL CALORIES FOR HEIGHT (OR TOTAL CALORIES FOR THE EXPECTED WEIGHT)¹

Height cm	Total calories per hour		Height cm	Total calories per hour	
	Girls	Boys		Girls	Boys
48	5 6		92	28 4	30 2
50	6 6		94	29 0	31 3
51		6 7	96	29 5	31 5
52	7 7	7 3	98	30 1	31 9
54	8 9	8 3	100	30 6	32 7
56	10 3	9 3	105	32 1	33 5
58	11 6	10 8	110	32 6	34 6
60	12 9	12 5	115	35 2	36 5
62	14 3	13 1	120	36 4	39 0
64	15 5	15 0	125	39 3	41 3
66	16 8	16 3	130	41 1	43 5
68	18 0	17 5	135	44 0	46 0
70	19 3	18 8	140	47 1	48 5
72	20 4	20 0	145	50 3	50 8
74	21 5	21 3	150	53 9	53 8
76	22 5	22 3	155	57 8	57 5
78	23 3	23 5	160	61 5	61 7
80	24 2	24 6	165	64 3	65 4
82	25 0	25 5	170	66 0	69 0
84	25 8	26 5	175	66 5	71 7
86	26 5	27 5	180	66 7	75 0
88	27 2	28 5	190		79 2
90	27 8	29 3			

¹ Reproduced by permission of the author and publisher from Talbot F B Basal Metabolism Standards for Children Am J Dis Child 55 455-459 (March) 1938

The standards are calculated in calories per hour instead of calories per 24 hours as in the original. Since the height standard is based on a normal weight this can also be called expected weight

D Common Sources of Error and Precautions Necessary to Prevent Them—1 If the slope of the curve indicates an excessively high rate, a leak is probable. Check the fit of the nose piece and mouth piece with a wisp of cotton and if no leak is found block the external auditory canals with the finger tips and note whether there is a change in the slope of the curve. Perforated ear drums may result in a leak which is very difficult to detect if this possibility is not considered. Ideally, the physician should examine the ear drums and inquire about perforations before making the appointment. If a leaking ear drum is found block the external auditory canals with a cotton pledget inside a small square of rubber dam.

2 Test the apparatus for leaks at least once a week as follows. Set up the apparatus as for an estimation raise the float until it contains 3 to 4 liters of air and

then tightly plug the ends of the breathing tubes. Place a weight equal to about 100 grams on top of the float and allow it to remain for ten minutes. Run the recording apparatus for the first and last minute of the test. Note the temperature. A perfectly straight tracing (provided the temperature of the apparatus has remained constant) indicates that no leak has occurred.

3. Examine the rubber valves each week and lubricate the connection of the lower valve with the apparatus to prevent sticking.

4. Keep the inside of the breathing tubes clean. Lubricate the connection of these tubes with the apparatus to secure an air tight joint.

5. If the soda lime is inactive a low rate results. At the end of each day lift out the soda lime box and pour the soda lime into a jar or wide mouth bottle so that it can be sealed tightly from the air. Do not replace the box in the apparatus.

It is advisable to keep about 5 pounds of soda lime on hand most of the time. Wilson's non caking 4 to 8 mesh soda lime which leaves the air 80 per cent saturated with moisture is recommended. Haden's factors can not be used with accuracy with other types of soda lime. Before using a new batch of it, sift out the powdered material.

Since a smaller amount of soda lime must be used than with the original metabolor it is exhausted with fewer basal metabolic rate tests. Inefficient absorption of carbon dioxide may be indicated by unusually deep respirations or discomfort of the patient.

Test the efficiency of the soda lime frequently. At the end of the test cork the breathing tube as soon as removed from the patient. Attach rubber tubing to the petcock and bubble some of the air from the apparatus into barium hydroxide solution. a precipitate indicates carbon dioxide is not being completely removed. If for the first batch of soda lime a record is kept of the time during which it was in actual use for metabolism tests before it became unsatisfactory, it will not be necessary to test subsequent batches so frequently.

6. The clock may be tested occasionally against an accurate watch noting the time required for the pen to pass across 10 to 50 small squares on the record paper. If inaccurate it can be corrected by a regulator.

SECTION VI GASTRIC CONTENTS ANALYSIS

A Procuring Secretions—The large Ewald tube is best for all aspirations except in the fractional tests, because it is more easily passed and stomach contents are more easily aspirated through it. It is important to empty the stomach completely at each aspiration, some suggest placing the patient in several different postures to facilitate this because the stomach is a poor mixer, as shown by the fact that different fractions of the total contents taken as close together as possible will vary in composition.

1 *Introduction of the Stomach Tube*—The large tube is directly inserted by propulsion whereas the small tube is swallowed with the aid of gravity. First of all the patient should be assured that the passage of the tube can do no harm and should be instructed as to how he can best cooperate. He should be placed in a straight backed chair preferably with the back against the wall, his clothes should be protected by towels or a large rubber apron, and his body should be tilted slightly forward. The tube should be sterilized and then chilled in a basin of cracked ice. The tube should be grasped near its end as one would a pen, and introduced far back into the pharynx, while the patient is asked to swallow. Then the patient should be instructed to breathe rapidly through the mouth while the tube is boldly pushed into the esophagus until the ring upon it reaches the incisor teeth thus indicating that the tip is in the stomach. After the tip of the tube passes the level of the cricoid cartilage there is very little discomfort. If the patient is extremely neurotic or possesses a pharyngeal hyperesthesia and does not have an idiosyncrasy to cocaine, a 2 per cent aqueous solution of cocaine hydrochloride may be sprayed into the throat before passing the tube.

2 *Vomitus*—This should be saved and sent to the laboratory for examination as described below because it may give all the information desired and thus eliminate the necessity of a test meal.

3 *Fasting Contents*—Removal and examination of this material may give the desired information. In cases having a large residuum it allows a more accurate interpretation of the test meal findings. It should always be done before the fractional tests.

4 *Ewald Meal*—It is recommended that this test be performed first in all patients requiring gastric analysis except those with pernicious anemia in whom the histamine test is done first. The original meal consisted of 35 grams of wheat bread or toast and 8 ounces of tea. Since bread or toast usually contains lactic acid and yeast, Dock has recommended the substitution of one shredded wheat biscuit. Graham crackers are more palatable with water and the residues

are more easily aspirated Tea, by reason of its tannic and gallic acid content, interferes with tests for blood and is, therefore, undesirable Bergeim demonstrated that water gives a similar gastric stimulation

Technic—One whole shredded wheat biscuit or, better, 3 graham crackers and 8 ounces of water are given on an empty stomach, preferably in the morning before breakfast The stomach is completely emptied at the end of 45 to 60 minutes, at least 30 to 50 cc should be obtained

5 **Histamine Test**—This was first put on a practical basis by Gompertz and Vorhaus¹ A great deal of work has been done with the method since then (see Chapter V for references) and recently a much smaller dose² of histamine has been recommended

Technic—The patient should be under as nearly basal conditions as possible when the test is done The small (Rehfuss) type of tube is swallowed and the fasting contents removed, then 0.25 cc³ of sterile 1-1000 solution of histamine⁴ is injected subcutaneously The stomach contents are completely aspirated at twenty and at thirty minutes from the time of injection and the volume and total acidity of the three samples are determined As the free acid is practically the same as the total acidity, it need not be estimated

6 **Riegel Meal**—This type of meal is the best test for slight degrees of impairment of total gastric function It should not be given until severe obstruction or impaired motility has been eliminated by an Ewald meal or fluoroscopic examination or both The test is best done after a fasting period, but may be done at other times, if the residuum is first aspirated The meal originally consisted of 400 cc of soup 200 grams of beefsteak, either two slices of bread or 150 grams of mashed potato and one glass of water An ordinary three course meal including the equivalent of 200 grams of beefsteak will give the same information Most information is secured if a small sample is removed at three hours for tests of acidity and observation of the progress of digestion, and if the stomach is emptied at six or seven hours to note whether any food residue remains

7 **Stasis Meal**—This should be given when impaired motility or obstruction to the outlet is suspected and has not been demonstrated by the Ewald meal or by

¹ Gompertz L. M. and Vorhaus M. G. Studies on the Action of Histamin on Human Gastric Secretion J Lab and Clin Med 11 14-21 (Oct) 1925

² Gompertz L. M. and Cohen W. The Effect of Smaller Doses of Histamin in Stimulating Human Gastric Secretion Am J Med Sc 177 59-64 (Jan) 1929

³ The older methods specified 0.1 or mg of histamine hydrochloride per kilogram of body weight but these doses usually produce a marked reaction in the patient (flushing of the face, headache etc) Gompertz has shown that a total dose of 0.25 mg will in all cases produce a definite response without the disagreeable reactions Although the response is not as great as with the larger doses it is not decreased in direct proportion Practically all of the work on responses to this test has been done with the larger doses but the curves obtained with either are similar in type and the results are therefore comparable

⁴ Burroughs and Wellcome's Ergamine is satisfactory

fluoroscopy Various meals are used, a satisfactory one consists of 2 ounces of half cooked rice (boiled 15 to 20 minutes) and 12 well chewed raw raisins. It is given in place of the regular evening meal and the stomach contents aspirated, preferably with the aid of washing in the morning before breakfast. The residuum is examined for food remnants.

8 Sippy Pumpings—These are samples removed during the Sippy treatment of peptic ulcer as a check on the alkali administration. They are usually removed at 4:30 P. M. and one half hour after the last dose of alkali in the evening and are tested for free acid. It is especially important to indicate the exact time of withdrawal of the sample. The volume should also be noted.

9 Alcohol Meal (Cheney)—Give 50 cc. of 7 per cent ethyl alcohol and aspirate at 45 minutes or by the fractional method.

10 Fractional Aspiration—A small tube of the Rehfuess type is swallowed by the patient, the fasting contents are removed. With the tube left in place, an Ewald or alcohol meal or injection of histamine is given and 10 cc. samples are removed at 10 minute intervals until the stomach is empty.

B Examination of Samples—**1 Gross Examination**—(a) **Color**—This should be accurately described. The fluid portion of normal gastric contents has a faint yellow color. Fresh blood imparts a red color, but older blood, due to reaction with the acid, has a dark color resembling coffee grounds. Bile produces a yellowish or greenish discoloration which is normally present.

(b) **Mucus**—This is recognized from its slimy appearance when the fluid is poured from one vessel into another. A small amount is normally present in aspirated contents, whereas a large amount is present in vomitus. Report the amount present (1+ to 4+).

(c) **Food Residues**—These may be visible grossly, but should be checked microscopically. Record the nature and approximate amounts of such remnants.

(d) **Volume**—This should always be measured before any material is withdrawn for tests.

2 Microscopic Examination—This should be made on the original unstrained material and should first be done with a subdued light and the 16 mm. or, better, 8 mm. objective. Pus (rare), blood, and bacteria should be identified with the higher power lenses. Epithelial cells, starch granules, a few bacteria, and occasionally yeast cells are normally present. Undigested food remnants, red cells, pus cells, sarcinae, and excessive numbers of yeast cells and bacteria are pathologic. A drop of diluted Lugol's solution will stain starch granules blue and 1 per cent aqueous methylthionine chloride (methylene blue) will stain bacteria. Gram's stain may aid in differentiating bacteria. Pus cells and red cells have the same appearance as in urinary sediments. Yeast cells are smooth, colorless, highly refractile.

oval cells a little smaller than a leukocyte, they stain yellow to brown with iodine solution and may show budding. Sarcinae are small spheres arranged in cuboid groups, often compared to cotton bales, they usually occur in clumps. Bacteria appear as elsewhere. The Boas Oppler bacillus is the only one of special significance, it is a long broad, gram positive rod, usually occurring in chains. They should be looked for in all cases of achlorhydria (see Fig. 16)

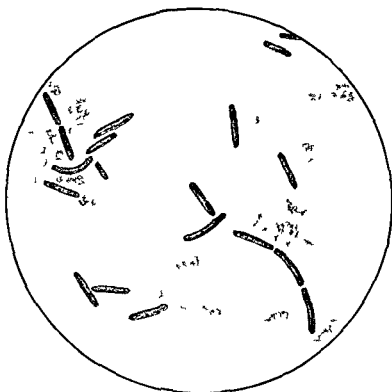


FIG. 16 —Boas Oppler bacilli in gastric contents

3 Chemical Examination—The stomach contents should be centrifugated or strained through gauze, not filtered, before this part of the examination is begun

(a) *Free Hydrochloric Acid* —(1) Congo red test This is merely a qualitative test and does not indicate the amount of free acid present. It may be done as a preliminary test, but is not necessary. A positive test is the production of a deep blue color when a drop of the gastric contents is placed on a strip of Congo red paper or a few drops are added to a dilute solution of the dye. The reaction occurs only at a low pH indicating a high degree of acidity which can not be produced by the slightly ionized organic acids.

(2) Boas' test This test is slightly less delicate than the dimethyl qualitative test but is more reliable since it reacts only to free hydro

chloric acid It should be used as a qualitative check on any "dimethyl" titration giving a clinical figure below 20

(a) Principle The reagent contains resorcinol and cane sugar, free hydrochloric acid plus heat causes the hydrolysis of the sugar with the formation of levulose and dextrose, levulose and resorcinol react in the presence of hot hydrochloric acid to produce a red color (Seliwanoff's levulose test)

(b) Technic In a porcelain dish mix two drops of gastric contents and Boas' reagent and slowly evaporate to dryness over a small flame, taking care not to scorch the sugar The appearance of a rose red color, which soon fades, shows the presence of free hydrochloric acid

(c) Reagent (Boas') Dissolve 5 grams of resublimed resorcinol and 3 grams of cane sugar in 100 cc of 50 per cent alcohol The reagent keeps well, which makes it preferable to Günzburg's reagent

(3) Dimethyl test (Topfer's) This is the most practical test for free acid but as the indicator reacts with other acids than hydrochloric, clinical figures below 20 must be checked by the Boas' test

(a) Principle This dye gives a red color in solutions of high acidity, the color diminishes as the pH of the solution approaches 4.0 and changes to pure yellow at about pH 4.3

(b) Technic Into a beaker or titration flask measure exactly 5 cc of strained stomach contents and add one drop of the indicator (Topfer's dimethyl) A cherry red color denotes the presence of free acid Now add N/10 sodium hydroxide from a burette until the red color disappears, leaving a pure canary yellow¹ color, read the burette and proceed as directed under "total acidity"

The presence of free hydrochloric acid should be checked by the Boas' method if this titration gives a clinical figure below 20 If the Boas' test is negative, report free hydrochloric acid *absent*, no matter what the dimethyl titration A clinical figure under 30 is an indication for a lactic acid test

Calculation Multiply the cc of hydroxide used for titration by 20 (i.e., $100\frac{2}{5}$) to convert to terms of 100 cc if using N/10 alkali, the result is cc of free N/10 hydrochloric acid per 100 cc of stomach contents or the clinical figure for free acidity The per cent of hydrochloric acid may be calculated by multiplying the acidity figure by 0.00365

(c) Reagent Topfer's dimethyl This is a 0.5 per cent alcoholic solution of dimethylaminoazobenzol

¹ Since this end point is not sharp and appears gradually beginners are likely to secure erroneous results with practice the end point is easily determined

(b) *Total Acidity*—This is made up of free and combined hydrochloric acid, acid salts, and, in some pathologic conditions, organic acids

(1) Principle The indicator used is phenolphthalein. Since its end point is on the alkaline side of neutrality, the results obtained by titration represent the total acidity of the solution (acid phosphates are converted into alkaline phosphate, e.g., disodium phosphate)

(2) Technic When the end point for free hydrochloric acid is reached, as described above, add one drop of 1 per cent alcoholic phenolphthalein solution to the mixture or this may be added at the start, and continue the titration to the characteristic phenolphthalein end point

Calculation The total cc of sodium hydroxide used for titration of the entire acidity, including the free acid, multiplied by 20 gives the total acidity of 100 cc of stomach contents in terms of N/10 acid. This is the clinical total acidity figure

(c) *Hydrogen Ion Concentration*—This is of importance only in connection with studies of peptic digestion. It is not a part of the routine examination. Colorimetric methods give satisfactory results and are the simplest to do. The method of Shohl and King is recommended.¹ Full directions accompany the standards and comparator

An approximate idea of the degree of deficiency of acid may be obtained by testing the gastric contents with Squibb's nitrazine paper. In pernicious anemia the gastric contents are often neutral or alkaline

(d) *Lactic Acid*—This acid occurs from the fermentation of carbohydrates in a stomach with hypochlorhydria or achlorhydria. Hence it should always be tested for in these cases if the "dimethyl" titration gives a clinical figure under 30. The Strauss ether ferric chloride test is satisfactory

(1) Principle Ferric chloride and lactic acid react to produce a yellow color which varies in intensity roughly in proportion to the amount of lactic acid present

(2) Technic To 5 cc of stomach contents, add 0.5 cc of N/1 hydrochloric acid and extract with 10 cc of ether. If a large test tube is used, close the tube and invert it repeatedly for 5 minutes, but do not shake in such a manner as to produce an emulsion. If emulsion occurs, separate it by centrifugation. With a large pipette, draw off the lower layer, then decant the ether into a clean test tube, leaving behind the few drops of aqueous liquid. If a separatory funnel is used, swirl the funnel in such a way that the liquids spread out in thin layers on the wall, finally draw off the watery layer and decant the ether into a test tube. If longer time can be allowed, put the liquids in a large flat

¹ Shohl A. T. and King J. H. Determination of the Acidity of Gastric Contents II The Colorimetric Determination of Free Hydrochloric Acid. Bull. Johns Hopkins Hosp. 31: 158-162 (May) 1920

bottle and lay it on its side, extraction occurs in an hour or two without shaking because the layer is very thin. Pour into a test-tube and separate the ether as in the first case above.

To the ether extract secured add 2 cc of distilled water and 4 drops of 2 per cent ferric chloride solution (old deeply colored solution gives a poor test, a trace of added hydrochloric acid restores the reagent) and shake. The solution acquires a canary yellow color if lactic acid is present.

(e) *Blood*—This may be tested for either by the ether extraction method as described under blood in urine (p 358) or directly on the residue strained out, as follows: pour a few drops of the ortho toluidine solution directly on the residue and then add a few drops of hydrogen peroxide. A positive test is the development of the characteristic greenish blue color.

(f) *Determination of Peptic Activity*—This is seldom done, but is of some value in checking the efficiency of alkali therapy in peptic ulcer cases and for the detection of achylia. The Mettè tube method with slight modifications is satisfactory. For more accurate methods, see reference 2 on page 126. Usually all the information desired is whether digestion has or has not taken place.

(1) *Principle* Small glass tubes containing coagulated egg albumin are placed in the solution to be tested and incubated. If pepsin and hydrochloric acid are present, the protein will be digested at each end of the tubes. The amount of digestion produced is a measure of the amount of pepsin present.

(2) *Technic* Place a Mettè tube in each of three narrow test tubes. To Tube 1 add 0.3 grams of *U S P* pepsin, 5 cc of water, and 3 drops of 10 per cent hydrochloric acid. To Tube 2, add 5 cc of strained gastric contents, and to Tube 3, add 5 cc of strained gastric contents and 3 drops of dilute hydrochloric acid. Place the tubes in an incubator at 37° C for 24 hours. After incubation examine the tubes to see if any digestion has taken place.

Tube 1 is the control and shows the effect of normal gastric juice. Digestion in Tube 2 indicates the presence of both pepsin and hydrochloric acid. Digestion in Tube 3 but not in Tube 2 indicates pepsin but no hydrochloric acid. No digestion in either Tube 2 or 3 indicates no pepsinogen or hydrochloric acid. This may be done in a quantitative way by accurately measuring the amount of albumin digested, but it is tedious and very inaccurate at best. Normal human gastric juice digests 2 to 4 mm of the albumin.

(3) *Apparatus* Mettè tubes. Mix and strain through cheese cloth the liquid portion of several egg whites. The mixture should be homogeneous and free from air bubbles. A number of thin walled glass tubes of 1 to 2 mm internal diameter should be cleaned, dried, and drawn out in a flame so that when broken they will be constricted at each end and 6 to 10 cm long. They should then be sucked full of the egg white and the tips sealed off in the flame. Coagulate the albumin by placing the tubes in a large bath of water at 85° C and allowing them to remain until cool. They keep for a long time. They are broken into pieces three fourths of an inch long when needed for use. The ends must be broken off squarely.

C Table 33 *Normal Values for Gastric Contents—Ewald Meal*

Volume	30 to 70 cc
Acidity (free HCl)	15 to 75 cc of N/10 acid per 100 cc
(total acidity)	30 to 90 cc of N/10 acid per 100 cc

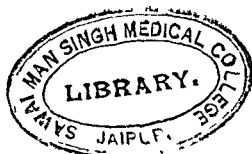
Histamine Test

Volume ¹	15 to 35 cc
Acidity ¹ (total)	90 to 125 cc of N/10 acid per 100 cc

¹ Volume and acidity of the last sample (30 minute aspiration)

Riegel Meal—At six hours the stomach should contain less than 100 cc of fluid of a consistency resembling puree

Mette Tube Test—Definite digestion of the albumin in Tube 2



SECTION VII EXAMINATION OF DUODENAL CONTENTS AND BILE¹

A Collection of the Specimen—Instruct the patient to come to the laboratory without breakfast

The duodenal tube may be passed more rapidly (Rousselot and Bowman) if 11 gauge piano wire having a bead of lead at one end is introduced into the Einhorn tube to stiffen it and the tube is passed at once into the stomach. Then withdraw the wire about 3 cm, turn the patient on the right side, pass the tube to the pylorus and check by fluoroscopy. Withdraw the wire to 5 cm from the tip and aid the passage of the tip through the pylorus by gentle manipulation under fluoroscopic observation. Withdraw the wire, inject 50 cc of saturated magnesium sulphate and maintain aspiration with a syringe for 60 minutes or until deeply bile stained fluid is obtained. Each 5 cc portion withdrawn is placed in a separate tube.

B Examination of the Contents—Note the character and intensity of the color. Normally one should obtain bile stained fluid which is viscid and slightly alkaline in reaction. Often the question of whether bile is obtainable or not is the only information desired. If gall stones are suspected, the most deeply stained portion should be centrifugated and the sediment examined for characteristic clear flat cholesterol crystals (Fig 12). Calcium bilirubinate appears as yellow to orange granules of irregular shape. If pancreatic function is to be determined, add 2 cc of a 1 to 10 dilution of the deepest colored sample to 2 cc of a 1 per cent soluble starch solution and incubate at 37.5° for 30 minutes. At the end of this time, add a drop of Gram's iodine solution. If any blue color remains, it indicates a deficiency of amylase. More accurate methods for testing for the presence of pancreatic enzymes, including tests for steapsin and trypsin, are given in the references.²

¹Lyon B B V Duodenal Tube Drainage of Biliary Systems In the *Cyclopedia of Medicine* 8 268-283 F A Davis Philadelphia 1933 This article and the references cited therein give in detail the technic and interpretation of the Meltzer-Lyon biliary drainage

²McClure C W, Wetmore A S and Reynolds, L New Methods for Estimating Enzymatic Activities of Duodenal Contents *Arch Int Med* 27 706-715 (June) 1921

Hollander E A Clinical Method for the Quantitative Determination of Pancreatic Ferments in Duodenal Contents *J Lab and Clin Med* 16 460-465 (Feb) 1931

SECTION VIII EXAMINATION OF FECES

The routine examination of feces should include a gross examination for color, odor, form, consistency, blood, mucus, and pus, a chemical test for blood, and a microscopic examination of a moist cover slip preparation for the presence of ova, parasites, and unusual amounts of fat or starch. Other tests are done only if specifically requested.

A Collection of Material—The stool should be passed into a clean vessel without admixture of urine and should be examined as soon as possible. Half pint paper cans¹ are satisfactory containers. When searching for motile forms of parasites it is especially important to keep the stool at body temperature all the time and to prevent excessive drying. When looking for amebae, flagellates, parasitic ova, worm segments or larvae, a fluid stool (e. g. the second stool after a saline cathartic) often has advantages. All specimens should be labelled with the patient's name in full, the exact time of collection and the tests desired.

B Gross Examination—**1 Color**—This should be accurately described in such terms as brown, yellow, clay colored, black and tarry, green, red, etc.

2 Odor—The normal odor, due to indol, skatol, and butyric acid is offensive, but not excessively foul. Describe the odor of the stool in such terms as normal, putrefactive, sour, extremely foul, etc.

3 Form and Consistency—Note whether the stool is formed or not and any marked deviations from the normal in shape (ball like, ribbon, etc.) and in diameter. Describe the consistency in such terms as fluid, semi fluid, mushy, soft, hard, of rocky hardness, etc. If gas bubbles are present, record the fact.

4 Blood—Large amounts of blood are usually evident grossly. Note the character as tarry, reddish, bright red, the amount on a scale of 1 to 4 plus and distribution as evenly mixed, partially mixed, only on the outside, etc.

5 Pus—Note the amount on a scale of 1 to 4 plus and the distribution.

6 Mucus—This is recognized by its slimy appearance and tenacious qualities. Record as 1 plus to 4 plus and note whether it is evenly mixed, only on the outside of the stool or if the specimen consists entirely of mucus.

¹ Obtainable from Menasha Products Co. Menasha, Wis.

7 Search for Gall Stones and Intestinal Worms—After the other examinations have been completed, filter the remaining fecal material through several layers of gauze, or better through a wire mesh strainer, with the addition of much water, using a wooden tongue blade to break up the firmer masses of feces. Care must be used because gallstones are easily broken. Gallstones may be readily identified by their faceted surfaces, their partial solubility if ground in a mortar with chloroform, and the fact that they float in water. Tests for the presence of cholesterol and bile pigments in the chloroform extract furnish positive confirmatory evidence but are rarely necessary. To test for cholesterol add to 2 cc of the extract, 1 cc of acetic anhydride and 0.1 cc of sulphuric acid. A green color develops within ten minutes if the test is positive. Filter the chloroform extract, evaporate in a dish and test for bilirubin with fuming nitric acid (see p. 356).

After the ingestion of large amounts of olive oil, small round or bean shaped masses of soap and fat appear in the feces, these are easily mistaken for gallstones and formed the basis for the old practice of giving large amounts of olive oil in cases of cholelithiasis. Enteroliths or intestinal concretions are occasionally, but rarely, seen. Seeds and fibers will also remain on the strainer but are of no significance.

Large segments of tapeworms and the adult forms of some other parasites are often visible when the stool is passed. Smaller segments and sometimes the adult small parasites may be found by washing the stool through a sieve. Any specimens which are not definitely identified by gross examination should be looked at with a microscope or high power magnifying glass. Identifying characteristics of worms, larvae and ova will be found under "E" in this section.

Sandlike granules or particles called intestinal sand may be found occasionally. They usually consist of calcium phosphate and calcium carbonate and are most common after ingestion of large amounts of milk or bananas. The presence of intestinal sand is of no diagnostic value.

8 Test for Diagnosis of Diarrhea and Constipation—Give the patient a capsule containing 1 gm of powdered charcoal or a capsule containing 0.3 gm of carmin and note the time. Ask the patient to bring in all stool specimens passed, in separate containers, noting for each the time at which it was passed. The time from the ingestion of the carmin to the time of passage of the stool which is colored by the dye indicates the time for passage through the gastrointestinal tract. A time over 48 hours indicates constipation and under 24 hours indicates diarrhea.

C Chemical Examination—**1 Reaction**—This may be tested with red and blue litmus paper or, better, with Squibb's nitrazine paper. Report the approximate pH if nitrazine paper is used or as strongly or faintly acid, alkaline or neutral.

2 Blood—A chemical test should be performed on all stools examined as well as an inspection for gross evidence of blood. The patient must be on a meat-free diet for at least 72 hours before a sample is collected for examination and positive tests due to ingested blood sometimes persist even longer. The patient should not eat meat, fish, broth or gravies and should be given either one gram of powdered charcoal or 0.3 gram of carmin, in capsules, with the first meat free meal so that the corresponding stool may be recognized and subsequent stools sent for examination.

Technic The stool may be tested directly with ortho toluidine solution and hydrogen peroxide but small amounts of blood may be missed and occasionally false positive tests may be secured. More accurate results are secured by the following technic. Mix some of the more suspicious portions of the feces with water in a mortar (extract first with ether if much fat is present) and then acidify with 50 per cent acetic acid, extract with ether and test the extract with a few drops of 1 per cent ortho toluidine in glacial acetic acid. Benzidine may be substituted for the ortho-toluidine but is somewhat less satisfactory. The ether extraction is just as necessary for accurate results with benzidine as with ortho toluidine.

Report blood as 1 plus if only a faint chemical test is secured, as 2 plus if a deep green color appears immediately in the chemical test but the stool is not grossly bloody or tarry, as 3 plus if the chemical test is positive and there is gross evidence of the presence of blood, and as 4 plus if the stool consists chiefly of bright blood or black tarry material giving a positive test for blood.

3 Bile Pigments and Urobilinogen—(a) *Schmidt's Test*—Rub up a small quantity of the stool with saturated (5 per cent) bichloride of mercury solution and let it stand for 24 hours. The urobilinogen normally present gives a red color, this will be absent in complete obstructive jaundice. Unchanged bilirubin which is normally absent gives a green color.

(b) *Quantitative Determination of Urobilinogen (Sparkman¹)*—(1) **Principle**—The urobilin is reduced to urobilinogen by ferrous sulphate and sodium hydroxide with the aid of heat. The aqueous solution of urobilinogen is separated from solid matter by filtration and after color

¹ See references on page 379

development by the action of paradimethylaminobenzaldehyde in acid is compared with an artificial standard in the colorimeter

(2) **Technic**—Weigh the stool specimen in its container and take up 5 gm of feces on a wooden tongue blade, determining the amount by difference in weight. Emulsify this in a mortar with 100 cc of distilled water, adding small portions of the water at a time, mixing and transferring to a 250 cc Erlenmeyer flask. Add a freshly prepared solution of 8 gm of ferrous sulphate in 40 cc of water and mix. Add 40 cc of 10 per cent sodium hydroxide slowly while mixing. Cork and shake vigorously. Incubate in a water bath at 50° C for 15 minutes. Remove and cool to room temperature and filter through Whatman No. 2 filter paper. To 5 cc of the filtrate in a test tube add 5 cc of distilled water, 0.3 cc of approximately 5N hydrochloric acid and 1 cc of aldehyde reagent. At the end of 5 minutes, read in a colorimeter against the artificial standard which most nearly matches it in color.

(3) **Calculation**—
$$\frac{\text{Reading of the standard}}{\text{Reading of the unknown}} \times \text{the factor} = \text{mg of urobilinogen per 100 gm of feces}$$
 Use the factor 630 if the strong standard is used, 185 if the intermediate standard is used, and 70 if the weak standard is used. The results are usually reported in this form but if the 24 hour excretion is desired, it may readily be calculated from the total weight of the 24 hour stool.

(4) **Reagents**—The reagents and standards are the same as those used in the quantitative determination of urobilinogen in urine. See page 357.

D Routine Microscopic Examination—This is a part of all routine stool examinations. Wooden tongue blades or applicator sticks which may be discarded are convenient for transferring the stool. Select portions, preferably those containing mucus, from several areas in the stool and emulsify a small amount with 0.9 per cent *sodium chloride* solution. Ideally this solution and the slides and cover slips to be used for this examination should be kept in the incubator so that they will be at body temperature. Place a drop of material on a slide and drop a cover slip over it which may be ringed with vaseline if desired. The low power or, better, the 8 mm objective should be used first and the higher powers later for positive identification.

Fats may appear as neutral fats, fatty acids, or soaps. Neutral fats form yellowish flakes or droplets which stain red with Sudan III. Fatty acids appear as flakes or needle like crystals, the flakes stain orange if a drop of a saturated solution of *Sudan III* in 70 per cent alcohol

is added to the mount. Soaps appear as amorphous flakes or rounded masses simulating parasitic ova and do not stain. Addition of acid and boiling liberates the fatty acids, which now take up Sudan III. Starch granules stain blue on addition of a drop of Gram's iodine solution. Muscle fibers are yellow, have square ends and transverse striations. Vegetable fibers are often spiral but may have various forms. Vegetable cells have a double contour and some (Fig 17, *f*) are easily mistaken for parasitic ova. Vegetable hairs often resemble parasitic larvae, but reveal on closer examination a central canal which extends the whole length. Pus cells, red cells, epithelial cells, and bacteria appear as elsewhere described. See Fig 17.

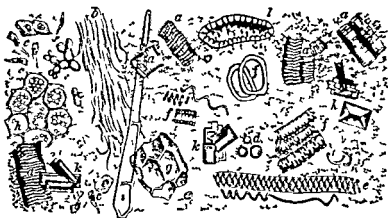


FIG 17—Normal Feces (Landois)

a Muscle fibers *b* tendon *c* epithelial cells *d* leukocytes *e*: various forms of plant cells among which are large numbers of bacteria between *h* and *b* are yeast-cells *k* ammonium magnesium phosphate

To test for pancreatic dysfunction, give the patient one-half pound of liver and a capsule containing 0.3 gm of carmin. Examine the marked stool microscopically for nuclei.

E Intestinal Parasites¹—1. **Collection of Specimens**—The stool must be brought to the laboratory as fresh as possible and must be kept at body temperature until the examination is completed if amebae or flagellates are to be looked for. Specimens containing mineral oil are unsatisfactory.

2. **Amebae**²—Rhizopoda or amebae are unicellular organisms progressing by pseudopodia and forming cysts. In examining for these, select pieces of mucus from freshly passed stool and emulsify in a drop of warm 0.9 per cent sodium chloride solution. Place next to it a drop

¹ Practice on the stools of dogs, rats or monkeys if suitable human material is not available.

² Magath, T. B. The Laboratory Diagnosis of Amebiasis. J. A. M. A. 103: 1218 (Oct 20) 1934.

of iodine-eosin stain so that when the cover glass is dropped in place the two drops will run together. Prepare the iodine eosin stain fresh daily by mixing 2 parts of 5 per cent potassium iodide in 0.9 per cent sodium chloride saturated with iodine, with 1 part of saturated eosin in 0.9 per cent sodium chloride. Examine at once on a warm stage under low power or, better, the 8 mm objective for objects resembling oil droplets and shift to high power for further identification. If the slides, cover glass, and stool are prewarmed, a warm stage is not essential. Look for the motile vegetative forms in the unstained portion. In the iodine eosin preparation, most structures except amebae and cysts stain with eosin, while the cysts stand out as slightly

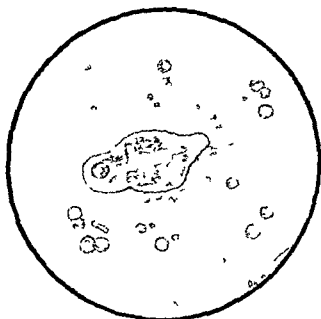


FIG 18—*Endameba histolytica* Containing Ingested Red Cell $\times 400$

vellow objects with brown granules if glycogen is present and the nuclei are more readily seen than in unstained preparations.

The *Endameba histolytica* is the only parasitic ameba of importance. It must be differentiated from the non pathogenic *Endolimax nana*, *Iodameba williamsii* and *Endameba coli* which frequently occur in the stools of normal individuals.

Motile stage. Look for these in an unstained preparation of mucus from fresh liquid stool obtained after giving a saline cathartic or by swabbing an ulcer through the sigmoidoscope. The major differentiating characteristics are given in Table 34. If an ameba progresses rapidly across the field, contains red cells or extends long finger like pseudopodia in the unstained preparation it is almost certainly *Endameba histolytica*.

TABLE 34 — DIFFERENTIAL DIAGNOSIS OF THE VEGETATIVE STAGES OF AMEBÆ

	<i>Endameba histolytica</i>	<i>Endameba coli</i>	<i>Endolimax nana</i>
Size	20 to 40 micra	20 to 30 micra	6 to 12 micra
Motility warm moist preparations)	Actively motile long and thrust out with explosive suddenness. It tends to progress rapidly across the field	Pseudopodia are Less actively motile are rounded distortions of outline Rarely moves from one place to another	Sluggish Pseudopodia are rounded distortions of outline Progresses slowly or not at all
Ectoplasm	Clear	Ground glass appearance	Ground glass appearance
Endoplasm	Finely granular	Ground glass appearance	Ground glass appearance
Ingested material	Often red cells or debris	few or no bacteria Bacteria and debris almost never red cells	Bacteria no red cells
Nucleus unstained	Pale hard to see	Distinct	Indistinct
Nucleus in iron hematoxylin preparations	4 to 7 micra A finely beaded ring of chromatin with a small central karyosome	4 to 7 micra A coarsely beaded chromatin ring with a large eccen- trically placed karyosome and often several chromatin masses between the karyosome and the ring	1 to 3 micra A very fine ring with all chromatin in large, irregular karyosome which almost fills the ring

DIFFERENTIAL DIAGNOSIS OF THE CYSTIC STAGES OF AMEBÆ

Size	7 to 15 micra	15 to 22 micra	7 to 9 micra
Form	Round	Round	Oval
Appearance unstained	Most refractile	Least refractile	Slightly refractile
Glycogen deposits (brown in iodine preparations)	Diffuse scanty	Diffuse More abundant in early stages	Absent or in a single mass
Number of nuclei ¹	1 to 4 usually 4	1 to 20 usually 8	1 to 4, rarely 8
Chromidia ²	Large bar forms common	Rare	Absent

¹ Morphology is the same as in the adult forms. They are seen well only in iron hematoxylin preparations though nuclei can be seen and counted by careful focusing in iodine preparations

² Dark staining chromatin masses outside of the nuclei

(Fig 18) The characteristics of the nucleus in iron hematoxylin stains are conclusive

Cystic stage Look for these in stained preparations from formed stools when active forms have been few or atypical or when the patient is thought to be a carrier. The large discrete spherical mass of glycogen seen best in the iodine preparations differentiates *Iodameba williamsii* (buetschli) from the other forms. The number of nuclei may be counted by careful focusing and if over four proves that the cyst is not that of *Endameba histolytica*. The chromatin distribution as seen in the iron hematoxylin preparations is the conclusive criterion for identification.

Do not confuse these with the more oval cysts of the intestinal flagellates which are frequently present but rarely cause symptoms. Cysts of amebae and flagellates are most satisfactorily differentiated with iron hematoxylin stains, the technic (Kofoid) for which is as follows:

Make a thin smear of the feces with a paste brush (if not albuminous, add a little serum or egg white). Immerse before it dries in Schaudinn's solution (*do not let the film dry at any time during the staining process*). After 30 minutes or longer, immerse successively in the following: iodine alcohol for 5 minutes, 70 per cent alcohol for 5 minutes, 50 per cent alcohol for 5 minutes and water for 2 minutes. Rinse with distilled water. Stain as follows: immerse in 2 per cent iron alum solution at 30° for 10 minutes, rinse with tap water for 5 minutes, rinse with distilled water.¹ Immerse in hematoxylin solution at 30° for 10 minutes, rinse with water, differentiate² in iron alum and wash well with running water for 15 minutes. Dehydrate and clear by using the following: 50 per cent alcohol for 5 minutes, 70 per cent alcohol for 5 minutes, 90 per cent alcohol for 5 minutes, first absolute alcohol for 5 minutes, second absolute alcohol for 5 minutes, first toluol for 5 minutes, and second toluol for 5 minutes. Mount in balsam thinned with toluol.

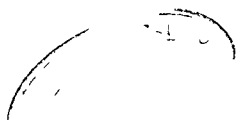
A practical point is to allow the slides to remain in the 70 per cent alcohol until several have accumulated and run all through at one time.

Reagents (1) Weigert's hematoxylin is the best. Make a 10 per cent solution of white crystals in absolute alcohol. Let it ripen for 3 months in sunshine. The ripening can be hastened by adding potassium permanganate until it is a wine red color. The solution used for staining, made fresh each day, consists of 1 part of stain to 19 parts of water.

(2) Schaudinn's solution. Mix 2 parts of saturated aqueous (6 per cent) mercuric chloride solution with 1 part of absolute ethyl alcohol.

¹ At this stage the smear may be dried and stained with Wright's stain as directed on p. 477 (Tsuchiya).

² This is the most exacting part of the process and requires experience for good results. Dip the slides into the solution and immediately place under the microscope and examine the nuclei. Repeat the process until the nuclei attain the proper appearance. It usually takes from a few seconds to a minute or more. The color should be a grey blue. If too dark, replace in iron alum and if too light, replace in the hematoxylin.



Ova of the Common Intestinal Parasitic Worms

All are shown at a magnification of 400 to 500 diameters. Note for all the doubly refracting sharply demarcated membrane which surrounds them and the tendency to brownish staining by the urobilinogen of the feces.

(1) *Necator americanus* ovum. The characteristic features are the mulberry like central portion surrounded by a clear zone. This central portion may be divided into 4, 8, 16 or more subdivisions.

(2) *Diphyllobothrium latum* ovum. The characteristic feature is the operculum which may be seen by careful focusing and the large size with regular oval contour. Some specimens may show partial or complete separation of the operculum and this may sometimes be produced by pressing on the cover glass forcing out some of the contents of the ovum.

(3) *Ascaris lumbricoides* ovum. These ova show outside of the sharp double membrane an irregular albuminous envelope which has a wavy outline and may be partially or totally lost.

(4) *Enterobius vermicularis* ovum. These ova are more often tightly packed inside of the worm itself or in scrapings from the perianal skin or under the finger nails than free in the stool. By pressure on the cover glass they may be extruded from the worm and their morphology recognized. Note the clear area separating the central portion from the outline and the characteristic flattening on one side of the otherwise elliptical membrane.

(5) *Hymenolepis nana* ovum. The internal structure of this ovum can hardly be mistaken for any other.

(6) *Trichuris trichiura* ovum. The characteristic projections at either end and the clear cut structure make this the easiest of all ova to identify.

(7) *Taenia saginata* ovum. The characteristic feature of these ova is the radial striation of the outer rim and the oval outline. The ova of *Taenia solium* are almost identical in appearance except that they are more nearly spherical. The ova of *Taenia solium* are so rarely found in this country that it is a fairly safe rule when ova resembling this are found to call them ova of *Taenia saginata*.



1



2



3



4



5



6



7

(3) Iodine alcohol 70 per cent alcohol made by dilution from 95 per cent alcohol saturated with iodine crystals

(4) Iron alum 2 per cent solution of ferric ammonium sulphate

3 **Flagellates and Ciliates**—Examine a moist cover slip preparation of fresh, warm fluid stool, obtained by catharsis if necessary. These parasites are rather common but only rarely produce active symptoms. The mature forms progress rapidly across the field in unstained specimens but the morphological details necessary for their identification are best seen with dark field illumination after their activity has somewhat diminished. The more important varieties with their chief differential characteristics are as follows

(a) *Trichomonas hominis*—These are 10 to 15 micra in length. They are pear shaped with 4 flagellae of equal length at the blunt anterior end and an undulating membrane on one side. A similar or possibly identical, organism, called the *Trichomonas vaginalis* is frequently found in the vaginal secretions in patients with leukorrhea.

(b) *Chilomastix mesnili*—They vary in length from 13 to 24 micra. This is a pear shaped organism having no undulating membrane and only 3 anterior flagellae. Posteriorly it ends in a narrow tail like process.

(c) *Giardia lamblia*—These are 12 to 20 micra in length, pear shaped with a depression at one side of the anterior end about which are 3 pairs of flagellae. The posterior end terminates in a pair of flagellae.

(d) *Balantidium coli*—These are oval, covered with cilia, actively motile and so large (50 to 70 by 70 to 100 micra) they can scarcely be confused with other parasites.

Ameba and flagellates may be cultured¹ but this is not as yet a practical method for diagnosis except in large laboratories.

4 **Intestinal Worms or Entozoa**—There are three classes the tape worms or cestodes, the round worms or nematodes, and the fluke worms or trematodes. The diagnosis is made by finding the whole worm or a portion of it on gross inspection or by finding ova or larvae on microscopic examination. It is often necessary to concentrate the ova if search of a direct smear is unsuccessful.

Recommended concentration technic Select several pea sized portions from different parts of the stool mix in a test tube with 5 cc of a 1 to 4 dilution of anti formin (sodium hydroxide 5 to 10 per cent in 10 per cent sodium hypochlorite), and warm but not to boiling. Let it stand until cold add 2 cc of water and shake with 5 cc of ether. Strain through one layer of gauze into a centrifuge tube and centrifugate one minute. Four distinct layers form with the ova at the bottom. Pour off the fluid if much fecal matter remains in the sediment repeat the centrifugation after treating with dilute (1 to 3) hydrochloric acid and more ether.

Flotation concentration method Mix a large sample of the stool with about twice its volume of saturated sodium chloride solution in a 1 inch test tube. Cork

¹ Kofoid C. A. and McNeil Ethel. The Advantages of Locke's Blood Medium in the Culture of Parasitic Protozoa of the Digestive Tract. Am J Hyg 15 315-317 (Jan) 1932

and shake vigorously until all particles are broken up. Let stand in an upright position for 1 hour. Touch the surface of the liquid with the mouth of a $\frac{1}{2}$ inch test tube and transfer the adherent film to the surface of a slide and examine microscopically. This method is unsatisfactory for operculated ova but is especially good for concentration of hookworm ova.

Locate ova under low power or, better, the 8 mm objective and identify them under the high power in a moist cover slip preparation or in a film of fecal matter dried on the slide and covered with oil. All ova have a *sharp outline with double refracting membranes*. See Fig 19 and Plate VIII for the morphology. The identification can be learned only by controlled practice. It is a fairly safe rule that if in doubt as to whether an object in the stool is a worm or ovum, it is not. The descriptions given below are for reference only, and need not be memorized. More detailed descriptions will be found in special works on parasitology. Report the form found and the stage as adult complete segments, larva, ova and approximate number on a scale of 1 to 4 plus.

(a) *Cestodes* (tape worms) —These are segmented, hermaphroditic, ribbon shaped worms and have no alimentary tract. The adult form and the larval form usually infest different hosts. Segments of tape worms are called proglottides.

(1) *Taenia saginata*, the beef tapeworm (common)

Adult. This occurs in the small intestines of man. It is greyish white, ribbon shaped, may be 4 to 8 yards long, and is divided into thousands of segments about 5 mm wide and 20 mm long. Portions may break off and be found in the stool or the whole worm may be passed after treatment. It is very important to find the head. Use the sieve technic if necessary. If the head remains it will regenerate the whole worm. The head end is more slender 1-2 mm in diameter, than the rest and is surmounted by 4 suckers, but has no hooks. To identify, a segment should be crushed between glass slides and it will be seen that the uterus has 15 or more arms.

Ova. These are passed in enormous numbers. They measure 35 to 40 micra by 20 to 30 micra, have a definite rim or shell with radial striations and are stained brown by the bile pigment in the feces.

Larval form. This occurs in cattle. The infestation occurs from eating insufficiently cooked beef from infected animals.

(2) *Taenia solium*, the pork tapeworm (rare)

Adult. This is similar to the beef tapeworm, except that it is shorter, that the head is surmounted by a crown of 24 to 28 hooks and that the uterus as seen in crushed segments has fewer arms. The segments measure about 5 by 10 mm.

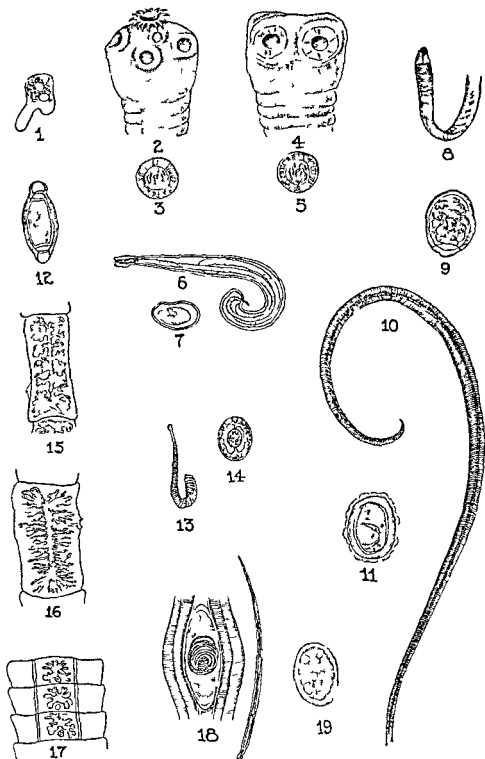


FIG 19—Parasites and ova 1 *Endameba histolytica* ($\times 200$) 2 and 3 *Taenia solium* head ($\times 10$) and ovum ($\times 400$) 4 and 5 *Taenia saginata* ($\times 10$) and ovum ($\times 400$) 6 and 7 *Oxyuris vermicularis* ($\times 10$) and ovum ($\times 200$) 8 and 9 *Diphyllobothrium latum* ($\times 5$) and ovum ($\times 200$) 10 and 11 *Ascaris lumbricoides* ($\times \frac{1}{2}$) and ovum ($\times 200$) 12 to 11 redrawn after Hemmeter 12 *Trichuris ovum* ($\times 400$) 13 and 14 *Hymenolepis nana* ($\times 1$) and ovum ($\times 400$) 15 Proglottis of *Taenia solium* ($\times 2$) 16 Proglottis of *Taenia saginata* ($\times 2$) 17 Proglottis of *Diphyllobothrium latum* ($\times 2$) 12 to 17 redrawn after Schmidt and Strasburger 18 *Trichinella spiralis* also coiled in muscle ($\times 50$) (after Claus) 19 Ovum of *Uncinaria americana* ($\times 200$) Ova 7 14 and 19 are colorless 3 5 9 11 and 12 are yellow to brown in color

Ova These are indistinguishable from those of *Taenia saginata*. They tend to be more spherical.

Larval form These occur in the hog, and infestation results from eating partially cooked pork.

(3) *Hymenolepis nana* the dwarf tapeworm

Adult This occurs in man and may also be found in rats and mice. It is very small, a few mm. to 5 cm. in length and $\frac{1}{2}$ mm. in breadth. The head contains 4 suckers and a rostellum of 24 to 30 hooks.

Ova These are passed intermittently and are 16 to 34 micra in diameter. They have a characteristic appearance. The small central part does not stain.

Larval stage This probably occurs in man also.

(4) *Diphyllobothrium latum*, the fish tapeworm

Adult This occurs in the small intestine of man and dogs. It is even larger than *Taenia saginata* or *solium*. It may be 40 feet long. The head has no hooklets, but a groove in its lateral surface. The segments show a brownish rosette shaped uterus in the center and are usually broader (10 mm.) than they are long (5 mm.).

Ova These are characteristic (70 micra by 45 micra), oval, with a cap or operculum at one end and mulberry like brown staining contents.

Larval form This occurs in fish.

(5) *Taenia echinococcus*, the dog tapeworm

Adult form This occurs in the intestine of dogs. It is 2 to 8 mm. long and usually has only 3 segments. The head shows 4 suckers and a crown of hooks.

Larval form This occurs in man, sheep, ox, and pig. The ova are swallowed and the larvae wander out of the intestine through the lymph channels, usually to the liver where they form cysts with daughter cysts inside. They may be present in almost any organ. The cysts removed at operation are identified by finding characteristic hooklets on microscopic examination of the sediment from the centrifugated cyst fluid. These hooklets have in rare instances been found in the sediment after centrifugating spinal fluid in cases involving the central nervous system.

Blood serum may be collected when such cysts are suspected and sent to a large laboratory for performance of a complement fixation test for echinococcus disease.

(b) *Nematodes or Round Worms*—These have an alimentary tract, but no segments, are cylindrical and taper. They are not hermaphroditic and usually have no intermediate hosts. The worms or ova may be found in the stool.

(1) *Ascaris lumbricoides* or large round worm

Adult The female is larger, 20 to 45 cm long and about 5 mm thick. The male is 15 to 17 cm long and 3 mm thick.

Ova These measure 60 by 45 micra. They have a thick shell. The center is stained yellow and in perfect specimens has a wavy albuminous membrane about it. This is, however, often partially or completely absent. Infertile ova are less regular in outline and harder to identify.

(2) *Enterobius (Oxyuris) vermicularis*, also called the pin worm, seat worm, or thread worm. It is often seen on the surface of the stool as a white, thread like object 5 to 10 mm long and may be identified

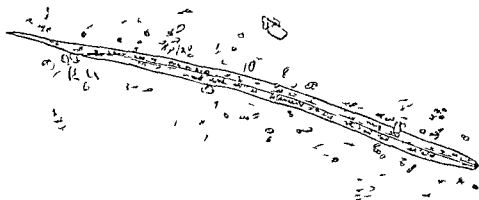


FIG 20—*Strongyloides Stercoralis* Larva $\times 300$

under the microscope by squeezing out some of the ova by pressure on the cover slip.

Ova These measure about 50 by 20 micra. These have a small oval center which does not stain, and are more easily found in the scrapings from under the finger nails or from the perianal folds than in the stool.

(3) *Strongyloides stercoralis* Larvae instead of ova appear in the stools. Under the low power of the microscope they are readily recognized in a moist preparation by their active thrashing motion. They are 250 to 500 micra long and are transmitted by contaminated water or vegetables. Fruit hairs and peach fuzz may resemble them but are non motile and the central canal extends to the tip instead of ending on one side as in the larvae (Fig 20). The larvae also show an oval structure about 30 micra long just posterior to the middle.

(4) *Trichuris trichiura*, *Trichocephalus dispar* or whip worms

The adults are thread like and about 3 to 5 cm long and are rarely seen in the stools.

Ova They measure about 50 by 20 micra. These are oval, brown, and have characteristic button like nodules at either end.

(5) *Necator americanus*, uncinaria americana or American hookworm

Adults are about 1 cm long They do not appear in the stools, but are found in the upper part of the small intestine

Ova These measure about 40 by 70 micra, and resemble mulberries in the appearance of the brown staining internal segments which may be 2, 4, 8 or more in number

(6) *Ankylostoma duodenale*, European or Old-World hookworm This is similar to the above, but the adults are larger and the eggs smaller (35 by 55 micra)

(7) *Trichinella spiralis*

The adult male is about 1.5 mm long and the female 3.4 mm They infest man, the rat, and the hog Man is infected by eating improperly cooked pork

Larvae (not ova) These burrow through the intestinal wall and are found coiled up in cysts in muscle removed for biopsy The removed tissue may be crushed between glass slides and examined with a 10 X magnifying glass or the low power microscope or, better, a good sized piece of muscle may be digested with pepsin and approximately N/10 hydrochloric acid and centrifugated and the sediment examined for larvae They are about 1 mm long Occasionally they may also be found from the sixth to the twenty-second day after infestation in the spinal fluid or in blood after laking with 5 volumes of 2 per cent acetic acid on centrifugation They rarely appear in the stool However, if a saline cathartic is given during the early stages of the disease, larvae may often be found in moist cover slip preparations of the stool

(c) *Trematodes or Fluke Worms*—These possess an alimentary tract are leaf shaped, hermaphroditic and unsegmented They are more common in animals and in organs such as the liver or bladder than in the bowel of man

(1) *Opisthorchis felinus*, the cat fluke It occasionally infests man

Adults These are yellowish red and measure 10 by 2 mm They are usually found in the gall bladder and bile passages

Ova These measure about 30 by 11 micra They are oval, smaller than others and have a definite operculum at one end

(2) *Clonorchis sinensis* This is a common parasite of cats, dogs, and man in China and Japan

Adults They resemble the *Opisthorchis felinus* in shape and color but are slightly larger

Ova These are operculated and measure 25 to 30 by 15 to 17 micra

(3) *Fasciolopsis buski* This is common in India, China, and Japan It inhabits the duodenum of man, producing bloody diarrhea

Adult It is the largest of the flukes, measuring 30 by 10 mm

Ova These measure about 135 by 80 micra The operculum is very small

(4) *Schistosoma hematobium* (Bilharzia hematobia) This organism lives in the veins of the bladder producing inflammation and hematuria. The ova are discharged in the urine.

Adults Unlike other trematodes except *Schistosoma mansoni* and *japonicum*, there are two sexes. The male measures 14 by 1 mm, and has a longitudinal ventral groove in which the female lies. The female measures 20 by 0.25 mm and is cylindrical.

Ova These are 120 to 190 micra long by 50 to 70 micra broad and are oval, not operculated, with a terminal spine.

(5) *Schistosoma mansoni* (Bilharzia mansoni) This fluke lives in the portal vein and its tributaries, and the ova are found in the feces.

Adults These resemble the adults of *S. hematobium* but are shorter, the male being 10 to 12 mm and the female 12 to 16 mm in length.

Ova These are about the same size as those of *S. hematobium* but have a lateral instead of a terminal spine and are found in the feces, not in the urine.

SECTION IX HEMATOLOGIC METHODS

A Routine Examination—This consists of a red cell count, hemoglobin estimation, white cell count, differential count, study of a stained smear, calculation of the color index and a sedimentation rate determination. If anemia is found, the reticulocyte count and volume and saturation indexes should be determined.

1 Collection of Blood—(a) *Capillary Blood*—With this method pipettes, diluting fluids, and slides for smears must all be taken to the patient as well as lancet, cotton and alcohol. The finger or ear chosen for the puncture must be free from edema, congestion, or inflammation and the skin through which the puncture is made must be clean and dry. One should avoid using an ear on which the patient has been lying, or the finger of a hand that has been hanging over the edge of the bed. Alcohol is usually used to sterilize and clean the skin over the point of puncture, but time must be given for all excess to evaporate or the drop of blood will spread out in a thin layer and the proteins will be coagulated, making it impossible to draw the blood into the pipette. The puncture should be made by a quick stroke of a *sharp* lancet. Adjustable spring lancets are satisfactory or a good substitute may be made by sticking the blade of a cataract knife or Bard Parker lancet through a cork into the 70 per cent alcohol bottle so that it protrudes on the inside a distance corresponding to the desired depth of the puncture. The puncture should be sufficiently deep to cause a free flow of blood *without pressure or manipulation*. These precautions are absolutely essential to accurate results but are seldom observed, hence venous blood is greatly to be preferred.

(b) *Venous Blood*—Only a dry, not necessarily sterile, 10 cc syringe with sterile needle, a tourniquet and a corked test tube containing 2 mg of dry potassium oxalate per cc of blood to be drawn, need to be taken to the patient in addition to the cotton and alcohol. After sterilizing the skin over the cubital vein with alcohol, apply a tourniquet just tight enough to distend the vein and have the patient clench his fist, draw the skin tense over the vein with the thumb of the left hand and at once with a *quick* thrust of the needle enter the vein. Draw up the desired amount of blood by slow traction on the plunger. Release the tourniquet, have the patient release his fist, press a pledget of cotton moistened in alcohol firmly over the puncture and *quickly*

withdraw the needle from the vein. Have the patient or an assistant hold the pledget firmly over the puncture to prevent a subcutaneous hematoma. Rapidly *remove* the needle from the end of the syringe, since forcing blood rapidly through the needle may cause hemolysis, transfer the blood to the test tube containing oxalate, replace the cork in the test tube, turn it to a horizontal position and *shake it vigorously* by tapping the end of the test tube with the right hand. Wash out the blood from the syringe and needle with cold water at once as this is much easier to do than later after the blood has clotted.

2 The Advantages of Oxalated Venous Blood and a Uniform System of Hematologic Methods —(a) 1 *Large Number of Hematologic Methods May Be Done on Oxalated Venous Blood*

TABLE 35—HEMATOLOGIC METHODS WHICH MAY BE PERFORMED ON OXALATED BLOOD

	Accurate if done within
(1) Hemoglobin estimation	24 hours
(2) Red cell count	24 hours
(3) Platelet count	1 hour (?)
(4) Red cell volume	3 hours
(5) Color index	24 hours
(6) Volume index	3 hours
(7) Saturation index	3 hours
(8) Icterus index	4 hours
(9) Van den Bergh test	4 hours
(10) White cell count	24 hours
(11) Making the smear for differential count	1 hour
(12) Peroxidase test	3 hours
(13) Fragility test	3 hours
(14) Sedimentation rate	3 hours
(15) Reticulocyte count	24 hours
(16) Paul and Bunnell test ¹	24 hours
(17) Serologic tests for syphilis	24 hours
(18) Blood grouping	24 hours

¹ This and the following tests are better done on serum but occasionally oxalated blood is already available and the patient is not at hand.

¹ Osgood E E. Hemoglobin Color Index Saturation Index and Volume Index Standards (Based on the Findings in 137 Young Men). Arch Int Med 37 685-706 (May) 1926.

Osgood E E and Haskins H D. Relation Between Cell Count Cell Volume and Hemoglobin Content of Venous Blood of Normal Young Women. Arch Int Med 39 643-655 (May) 1927.

Osgood E E Haskins H D and Trotman F F. A Uniform System of Hematologic Methods for Use with Oxalated Venous Blood. J Lab & Clin Med 16 476-494 (Feb) 1931.

Wintrobe M M. Blood of Normal Young Women Residing in a Subtropical Climate. Arch Int Med 45 287-301 (Feb) 1930. This article gives the literature on the use of oxalated blood.

(b) *The Convenience of Doing the Methods Is Greater*—(1) Only a syringe, sterile needle, tourniquet, test tube containing oxalate, cotton and alcohol need be carried to the patient

(2) The estimations do not have to be made immediately and if there is a suspicion of error they can be repeated without the embarrassment of returning to the patient for more blood

(3) If blood is desired for a Wassermann coagulation time, or for blood chemistry tests, no extra puncture is necessary

(4) If during the study of the blood further hematologic work is deemed desirable it may be done on the same sample

(5) If an unusual or interesting blood picture is encountered, as many slides as are desirable can be made for future reference or for teaching purposes without again disturbing the patient

(6) If the smear is made and sent along for staining and examination, blood can be mailed considerable distances to a central laboratory for expert examination

(c) *The Accuracy Is Greater*—(1) Larger quantities of blood may be used for the hemoglobin estimation, insuring more accurate results

(2) Time is allowed for making measurements. The haste necessary to prevent clotting as when capillary blood is used, tends to cause inaccuracy

(3) Errors (which are avoidable, but seldom avoided) due to manipulation or constriction of the part, to local edema or congestion, common to the use of capillary blood are completely done away with. Drucker has shown that in order to get accurate results on capillary blood a sufficiently deep wound must be made to cause at least three drops of blood to flow without any manipulation whatsoever, and that the first two drops must be discarded. Anyone who has tried to train medical students or technicians knows how few of them actually observe these essentials for accurate counting. If these precautions are observed, counts agreeing with those on venous blood are obtained

(4) Duplicate estimations can be run on the same sample, which serve to increase accuracy, show up poor technic and poor methods, and allow one to form an opinion of the accuracy being attained

If the work is done by technicians questionable results can at any time be checked on the same sample of blood. By having them occasionally run a series of estimations on the same sample they learn their experimental error and can be encouraged to reduce it. New technicians or students can be taught to repeat results on the same sample until they consistently agree within the desired limits. Hematologic work can be sent to a central laboratory and its accuracy can occasionally be checked by dividing a well mixed sample between two tubes and either sending in one and examining the other or sending in both tubes with different labels and noting how the results agree

(d) *Normal Values Have Been More Carefully Standardized on Venous Blood*, and many who have compared it with capillary blood prefer it

(e) *It Is Less Disagreeable to the Patient*—(1) Venipuncture is less painful than puncture of the ear or finger.¹ Twenty of 28 students who were asked, expressed a preference for venipuncture to finger or ear puncture

¹ Root H F, Thompson J W and White R R. Some Relations Between the Concentration of Blood Corpuscles in Venous and Capillary Blood and the Blood Pressure of Diabetic Patients. J Lab and Clin Med 11: 406-412 (Feb) 1926

(2) One puncture suffices for serologic hematologic, and blood chemical tests

3 Answers to Objections to the Use of Venous Blood —(a) Venipuncture is not practical on all patients This is valid and the older methods should be used on certain children and obese patients with small veins

(b) The quantity of blood removed will harm the patient This is a myth If 5 cc of blood were withdrawn daily for ten days, and no regeneration occurred but the blood volume returned to its previous value, the hemoglobin percentage would theoretically be about 1 per cent lower than on the first day Normal daily variations far exceed this

(c) Patients object to it This is very rare The method has been in routine use in many hospitals, clinics and offices for eleven years with satisfaction to all concerned Patients objections to venipuncture have not prevented the steady increase in the number of hospitals and clinics in which a serologic test for syphilis is a routine procedure

4 Methods and Precautions for Using Venous Blood —(a) Prepare a month's supply of test tubes, containing 2 mg of dry potassium oxalate per cc of blood to be taken, by measuring into each, with a burette, 0.1 cc of 2 per cent potassium oxalate for each cc of blood to be taken and evaporating this to dryness in a drying oven or over a radiator Keep the tubes corked and in baskets labelled with the amount (5, 10, 20 cc) of blood for which they were prepared See Table 23, p 422 Five cc of blood is sufficient for the "routine" hematologic examination and 10 cc for a complete examination

(b) Draw blood from the vein by the usual technic, but the tourniquet should be released if more than two minutes are required for securing sufficient blood, since the red cell count begins to increase after three minutes of stasis

(c) Remove the needle from the syringe before running the blood into the oxalate tube as hemolysis will result if the blood is forced through the needle

(d) Cork the test tube, never use cotton, and at once shake *vigorously* by holding the test tube *horizontally* in the left hand and tapping the other end with the right hand

(e) The blood must be thoroughly mixed in this manner for 30 seconds immediately before samples are withdrawn for any test

(f) Samples should be taken directly from the test tube, not from blood poured out on a slide or watch glass

(g) The tube must be kept corked at all times when not in use

(h) The time limits noted (see p 459) should be observed if the most accurate results are desired, although as a rule a slightly longer time will not introduce clinical error

5 **Hemoglobin Estimations**¹—For small laboratories where less than 8 to 10 estimations per day are done, the Haskins Sahli² method is recommended. For larger laboratories, the Osgood Haskins³ method, as modified by the author is recommended.⁴

All hemoglobin estimations should be reported in grams per 100 cc and the method used should be stated. Otherwise the great variation (13.8 to 17.3) in the number of grams of hemoglobin taken as 100 per cent in different methods and the enormous differences in the accuracy of the methods will make correct interpretation of the results impossible.

In all acid hematin methods it is important that the directions call for heating the standard. Otherwise, the color continues to change. Some methods include a correction for time but it is difficult to make readings always at a constant time after the dilution is made and the process of full color development requires 24 hours at room temperature. Furthermore, time is not the only variable affecting the rate of color development. The concentration of acid and of hemoglobin and differences in room temperature also influence this rate of development. In all instruments depending on the Sahli principle it is essential that the tubes containing the standard and those containing the unknown have exactly the same internal diameter and that the pipette represents a volume exactly 0.01 of that at the 100 per cent mark on the calibrated tube. Errors as high as 25 per cent have been found in the calibration of some instruments on the market. It is prohibitively expensive for manufacturers to calibrate individually the solid glass rods or discs, whereas, it is perfectly feasible to spend several weeks accurately testing the color value of a liquid standard. So methods employing liquid standards are recommended for laboratories which are not equipped to recalibrate glass standards against a gasometric method.

(a) *Haskins Sahli Method*⁵—Haskins has prepared a standard solution for the Sahli apparatus that has the right color and is permanent. It contains only inorganic salts and has not changed in a period of sixteen years.

¹ Schwentker F. F. The Estimation of Hemoglobin. A New Hemoglobinometer. *J. Lab. and Clin. Med.* 15: 247-259 (Dec.) 1929. This article contains the most complete bibliography on hemoglobin methods yet published.

Haden R. L. Hemoglobin Standards. *Am. J. Clin. Path.* 3: 85-95 (Jan.) 1933.

² Haskins H. D. and Osgood E. E. Methods of Estimating Hemoglobin. *Northwest Med.* 25: 500-503 (Sept.) 1926.

³ Osgood E. E. and Haskins H. D. A New Permanent Standard for Estimation of Hemoglobin by the Acid Hematin Method. *J. Biol. Chem.* 57: 107-110 (Aug.) 1923.

⁴ See page 468.

⁵ The apparatus is obtainable from the Shaw Surgical Co., Portland, Oregon. Each apparatus is tested for accuracy of calibration of pipettes and graduated tubes.

(1) **Principle** A known amount of blood is treated with an excess of hydrochloric acid which converts the hemoglobin to acid hematin. The intensity of the brown color of the acid hematin is proportional to the amount of hemoglobin present and therefore, can be compared to a standard of known value. The reaction between the acid and the hemoglobin is not immediate, it requires at least 4 hours at room temperature to become complete. If the mixture is heated, the reaction is completed in 7 minutes at a temperature of 55 to 60° C. The temperature must never be higher because the blood proteins will be precipitated.

The permanent standard, when at 20° C, has the same color by transmitted light as a blood containing 13.8 grams of hemoglobin per 100 cc. when it is diluted exactly to the 100 mark in a Sahli tube.

(2) **Technic** Put N/5 hydrochloric acid in the graduated tube about to the 10 mark. Deliver exactly 0.02 cc. of blood with the Sahli pipette as quickly as possible into the solution and *mix at once* by tapping the tube vigorously. See that there are no clots. Draw the solution into the pipette twice, blowing it out into the tube, then rinse the tip with a drop of N/5 hydrochloric acid. Place the tube in water at 55 to 60° C. for seven minutes. Longer heating does no harm. In that length of time the full acid hematin color is developed. Cool the tube and dilute gradually with N/5 hydrochloric acid mixing after each addition of acid until it matches the standard. Use a clean copper wire with a loop at the end as a stirring rod. Make the reading from the graduated tube. Use a fraction of a drop of caprylic alcohol to cut any foam present. If in doubt as to the end point, add another drop of acid, mix and again compare. If it is now too pale the end point had been reached. Note the temperature of the room in the vicinity of the standard. If it is 20° C. no correction is necessary, at higher temperatures the standard becomes darker, due to increased hydrolytic dissociation and under estimation results. For each degree above 20° C. add one hundredth part of the Sahli reading and for each degree below 20° C. deduct one hundredth part. For example, with the standard at 22° C. an estimation reads 81 per cent, corrected it becomes 82.6 per cent.

The Haskins Sahli method easily gives results with less than 5 per cent error in routine work, when the specifications mentioned above are met.

(3) **Reagents** N/5 hydrochloric acid dilute 9 cc. of C.P. acid to 500 cc. with distilled water.

(b) *Osgood Haskins Method*—This is an acid hematin method, using an ordinary colorimeter and a permanent standard¹ which contains

¹ The permanent standard contains 32 grams of ferric sulphate and 80 mg. of chromic sulphate in 100 cc. of solution. It can not be produced by weighing out the constituents

only inorganic salts and has not changed in sixteen years. It is especially suited for use with oxalated venous blood, although blood from the finger or ear can be used. It is especially useful when many estimations are to be made in one day. It is comparable¹ to the much more difficult oxygen capacity or blood iron methods in accuracy but requires less than five minutes time per estimation.

(1) Principle The method depends on the conversion of hemoglobin to acid hematin as in the preceding method and matching with a permanent standard in a colorimeter.

(2) Technic Measure exactly 1 cc² of *well mixed* oxalated blood into a 100 cc volumetric flask³ containing about 40 cc of distilled water⁴. After laking has occurred, add about 50 cc of N/5 hydrochloric acid⁴ while mixing, and dilute to the mark. If there is foam, cut it with a drop of ethyl, not caprylic, alcohol. Mix thoroughly and pour part of the solution into a test tube. Heat the tube in a bath at 55 to 60° C⁵ (hot tap water) for 7 minutes (or longer), and cool. Estimate with a colorimeter, setting the permanent standard at 15 mm. When 3 or 4 readings have been made, take the temperature of the standard at once by inserting a clean dry thermometer in the liquid in the colorimeter cup.

Calculation In Tables 36 and 37 which are furnished also with the standard read the per cent in the proper temperature column.

and dissolving since ferric sulphate of uniform color value is not obtainable. Standard solution (checked by Dr. E. E. Osgood) can be secured from Hynson, Westcott and Dunning, Baltimore, or from the Shaw Surgical Co., Portland, Oregon.

¹ Dowden, C. W., McNeill, C. and McNeill, J. D. A Clinical Study of Blood Iron and Hemoglobin. *J. Lab. and Clin. Med.* 19, 362-371 (Jan.) 1934.

Andresen, Marjory I. and Mugaage, E. R. Red Blood Cell Values for Normal Men and Women. *Arch. Int. Med.* 58, 136-146 (July) 1936.

² It is desirable to have the pipettes graduated to contain 1 cc. to the tip for each can then be stood up in the 100 cc. flask to drain while the next pipette is filled and the water from the siphon bottle delivered through it into the flask thus rinsing out the remaining blood and insuring accurate measurements without the necessity of care in observance of the rate of flow and drainage time.

³ If less blood is available dilute 0.5 cc. in a 50 cc. flask. In marked anemia dilute 1 cc. of blood to 50 or 25 cc. and divide the calculated results by 2 or 4 respectively. If finger or ear blood is used measure exactly 0.05 cc. into exactly 2.45 cc. of water (previously measured into a series of test tubes which are kept tightly corked) rinsing the pipette as in the Sahli method when laked add exactly 2.5 cc. of N/5 hydrochloric acid.

⁴ The use of two overhead siphon bottles of 2 liter capacity to contain distilled water and N/5 hydrochloric acid speeds up the measurements. A 5 cc. variation is unimportant (the N/5 hydrochloric acid may be 45 to 55 cc.). A measuring device may be inserted into the siphon tract fit a length of 1 inch glass tubing with stoppers, a two hole stopper at the top connects with the siphon and with an air vent tube, a one hole stopper at the bottom connects with a glass tube (and rubber tube) for delivery. There must be a pinch cock above to control inflow and below for outflow. Graduate for 50 cc. (and 25) by measuring water into the tube and making marks with a file or grease pencil.

⁵ A constant temperature bath (55-58°) is desirable if a large number of estimations are being made.

TABLE 36—DETERMINATION OF PER CENT HEMOGLOBIN—(Continued)

Colorimeter reading mm	Temperature of the standard											
	15 5°	16 5	17 5	18 5°	19 5	20 5	21 5	22 5	23 5	24 5	25 5	26 5
13 7	77 0	78 4	79 9	81 4	82 9	84 5	86 1	88 0	89 9	91 8	93 7	95 2
13 8	76 4	77 8	79 3	80 8	82 3	83 9	85 5	87 3	89 2	91 1	93 0	94 7
13 9	75 8	77 2	78 7	80 2	81 8	83 3	84 9	86 7	88 6	90 5	92 4	94 0
14 0	75 2	76 7	78 2	79 6	81 3	82 8	84 3	86 1	88 0	89 9	91 8	93 4
14 1	74 6	76 1	77 6	79 1	80 7	82 2	83 7	85 5	87 3	89 2	91 1	93 8
14 2	74 0	75 5	77 1	78 6	80 1	81 6	83 2	84 9	86 7	88 6	90 5	92 9
14 3	73 5	75 0	76 6	78 1	79 6	81 1	82 7	84 4	86 1	87 9	89 8	91 6
14 4	73 0	74 5	76 0	77 5	79 0	80 6	82 2	83 8	85 5	87 3	89 2	91 1
14 5	72 5	74 0	75 4	76 9	78 4	80 0	81 6	83 2	84 9	86 7	88 6	90 5
14 6	72 1	73 5	74 9	76 3	77 8	79 4	81 0	82 6	84 3	86 1	88 0	89 9
14 7	71 6	73 0	74 4	75 8	77 2	78 8	80 4	82 0	83 7	85 5	87 4	89 3
14 8	71 2	72 5	73 9	75 3	76 7	78 2	79 8	81 5	83 2	85 0	86 8	88 7
14 9	70 8	72 0	73 4	74 8	76 0	77 6	79 2	80 9	82 7	84 3	86 2	87 9
15 0	70 2	71 6	73 0	74 4	75 8	77 3	78 8	80 4	82 2	83 9	85 4	87 4
15 2	69 1	70 6	72 0	73 4	74 9	76 3	77 7	79 3	81 0	82 7	84 3	86 1
15 4	68 4	69 7	71 1	72 4	73 8	75 3	76 8	78 4	80 0	81 6	83 3	84 8
15 6	67 4	68 8	70 1	71 4	72 8	74 3	75 9	77 5	79 0	80 6	82 2	83 8
15 8	66 5	67 9	69 2	70 4	71 9	73 3	74 8	76 4	78 0	79 6	81 2	82 8
16 0	65 8	67 1	68 4	69 7	71 0	72 4	73 8	75 4	77 0	78 6	80 3	81 9
16 2	64 9	66 2	67 6	69 2	70 1	71 5	72 9	74 5	76 1	77 7	79 4	81 1
16 4	64 1	65 3	66 8	68 0	69 3	70 6	72 0	73 6	75 2	76 8	78 5	80 1
16 6	63 3	64 6	65 8	67 1	68 4	69 7	71 2	72 7	74 3	75 8	77 4	79 1
16 8	62 6	63 8	65 2	66 5	67 8	69 1	70 4	71 9	73 4	74 9	76 5	78 1
17 0	61 9	63 1	64 4	65 6	66 9	68 1	69 6	71 0	72 4	74 0	75 6	77 2
17 2	61 1	62 3	63 6	64 8	66 1	67 4	68 8	70 2	71 7	73 2	74 7	76 3
17 4	60 3	61 6	62 9	64 1	65 3	66 6	67 9	69 3	70 8	72 3	73 8	75 3
17 6	59 6	60 9	62 2	63 4	64 6	65 8	67 0	68 5	70 0	71 5	73 0	74 5
17 8	59 0	60 2	61 4	62 7	63 9	65 1	66 4	67 8	69 1	70 7	72 3	73 8
18 0	58 5	59 6	60 8	62 0	63 2	64 5	65 8	67 0	68 3	69 8	71 4	73 1
18 2	57 9	59 0	60 2	61 3	62 5	63 8	65 0	66 3	67 7	69 1	70 6	72 3
18 4	57 2	58 3	59 6	60 7	61 9	63 1	64 3	65 7	67 1	68 5	69 9	71 4
18 6	56 6	57 7	59 0	60 1	61 2	62 3	63 5	64 9	66 3	67 7	69 2	70 6
18 8	55 9	57 0	58 3	59 4	60 6	61 7	62 7	64 1	65 5	66 9	68 4	69 9
19 0	55 3	56 4	57 7	58 8	59 8	61 0	62 0	63 3	64 7	66 2	67 7	69 2
19 2	54 7	55 8	57 1	58 1	59 2	60 2	61 3	62 6	64 0	65 5	67 0	68 4
19 4	54 1	55 2	56 3	57 4	58 5	59 7	60 9	62 2	63 6	64 9	66 2	67 7
19 6	53 6	54 6	55 7	56 8	57 9	59 1	60 3	61 6	62 8	64 3	65 5	66 9
19 8	53 0	54 1	55 2	56 3	57 4	58 5	59 7	60 9	62 1	63 4	64 8	66 2
20 0	52 5	53 6	54 6	55 7	56 8	57 9	59 1	60 2	61 6	62 8	64 1	65 6
20 4	51 5	52 5	53 6	54 6	55 6	56 7	57 9	59 0	60 2	61 5	62 7	64 3

opposite the average mm reading,¹ interpolating when necessary. Convert the per cent figure into grams per 100 cc of blood by using Table 43 (p 489).

An error of over 2 per cent is unusual in routine work and, with research care, it can be held within 1 per cent.

Instead of being heated the acid hematin solutions may stand at room temperature 24 hours, or even several days before being estimated.

¹ If the colorimeter reading is higher than 20.4 mm, divide it by 2 and look up the per cent in the table and then divide by 2. A better method is to make a fresh preparation of acid hematin diluting to 50 or 25 cc instead of to 100 cc, the calculated result is then divided by 2 or 4.

TABLE 37—HEMOGLOBIN TABLE FOR HIGHER TEMPERATURES

Colorimeter reading mm.	Temperature of the standard										
	27 5	28 5	29 5	30 5	31 5	mm	27 5	28 5	29 5 ^a	30 5	31 5
9 4	142 9	146 2	149 6	153 0	156 0	14 4	93 2	95 4	97 6	100 1	102 6
9 5	141 5	144 7	148 0	151 1	154 3	14 5	92 6	94 8	97 0	99 5	101 9
9 6	140 1	143 3	146 5	149 7	152 9	14 6	92 0	94 1	96 3	98 8	101 2
9 7	138 7	141 8	145 0	148 4	151 8	14 7	91 4	93 5	95 6	98 1	100 5
9 8	137 1	140 3	143 5	147 1	150 6	14 8	90 8	92 9	95 0	97 4	99 8
9 9	135 6	138 8	142 1	145 6	149 1	14 9	90 2	92 3	94 4	96 7	99 2
10 0	134 2	137 3	140 5	144 1	147 7	15 0	89 6	91 7	93 8	96 1	98 5
10 1	132 8	135 9	139 0	142 6	146 2	15 2	88 4	90 5	92 6	94 9	97 3
10 2	131 6	134 6	137 7	141 3	144 8	15 4	87 2	89 3	91 4	93 7	96 0
10 3	130 4	133 4	136 5	140 0	143 4	15 6	86 0	88 1	90 2	92 6	94 7
10 4	129 1	132 1	135 2	138 6	142 0	15 8	85 0	87 0	89 1	91 2	93 5
10 5	127 8	130 8	133 9	137 4	140 8	16 0	84 0	85 9	87 9	90 1	92 4
10 6	126 6	129 6	132 7	136 0	139 6	16 2	82 9	84 8	86 6	89 0	91 2
10 7	125 4	128 4	131 5	134 7	138 0	16 4	81 8	83 8	85 8	88 0	90 1
10 8	124 2	127 1	130 2	133 4	136 6	16 6	80 9	82 8	84 8	86 9	89 0
10 9	123 1	126 0	129 0	132 2	135 4	16 8	80 0	81 9	83 8	85 8	87 9
11 0	122 0	124 9	127 9	131 1	134 3	17 0	79 1	81 0	82 8	84 8	86 9
11 1	120 9	123 8	126 8	129 9	133 1	17 2	78 2	80 0	81 8	83 8	85 9
11 2	119 9	122 7	125 6	128 8	132 0	17 4	77 3	79 1	80 9	82 9	84 9
11 3	118 9	121 5	124 5	127 7	130 9	17 6	76 4	78 2	79 9	81 9	83 9
11 4	117 8	120 4	123 4	126 6	129 7	17 8	75 5	77 3	79 0	81 0	83 0
11 5	116 8	119 4	122 4	125 5	128 6	18 0	74 6	76 4	78 2	80 1	82 1
11 6	115 7	118 4	121 4	124 4	127 5	18 2	73 8	75 5	77 3	79 2	81 2
11 7	114 7	117 4	120 4	123 4	126 4	18 4	73 0	74 7	76 5	78 4	80 4
11 8	113 7	116 4	119 3	122 3	125 3	18 6	72 2	73 9	75 7	77 6	79 5
11 9	112 8	115 5	118 2	121 1	124 1	18 8	71 4	73 1	74 9	76 8	78 6
12 0	111 8	114 5	117 2	120 1	123 0	19 0	70 7	72 3	74 0	75 9	77 8
12 1	110 9	113 6	116 3	119 2	122 1	19 2	70 0	71 6	73 2	75 0	76 9
12 2	110 0	112 7	115 4	118 3	121 2	19 4	69 2	70 8	72 4	74 2	76 1
12 3	109 1	111 8	114 5	117 3	120 1	19 6	68 5	70 1	71 7	73 5	75 4
12 4	108 3	110 8	113 4	116 2	119 0	19 8	67 8	69 4	71 0	72 8	74 7
12 5	107 4	109 9	112 4	115 2	118 0	20 0	67 1	68 7	70 3	72 1	73 9
12 6	106 5	109 0	111 5	114 3	117 1	20 2	66 4	67 9	69 5	71 3	73 1
12 7	105 6	108 1	110 7	113 5	116 2	20 4	65 8	67 3	68 8	70 7	72 4
12 8	104 8	107 3	109 9	112 7	115 4	20 6	65 2	66 7	68 3	70 0	71 9
12 9	104 0	106 5	109 0	111 7	114 4	20 8	64 5	66 1	67 7	69 4	71 4
13 0	103 3	105 6	108 2	110 8	113 5	21 0	63 9	65 4	67 0	68 7	70 4
13 1	102 5	104 9	107 3	110 0	112 7	21 2	63 3	64 8	66 3	68 0	69 8
13 2	101 7	104 1	106 5	109 2	111 9	21 4	62 7	64 2	65 7	67 3	69 0
13 3	100 9	103 4	105 7	108 4	111 1	21 6	62 1	63 5	65 1	66 7	68 3
13 4	100 2	102 6	105 0	107 6	110 3	21 8	61 5	63 0	64 5	66 2	67 7
13 5	99 6	101 9	104 2	106 8	109 4	22 0	61 0	62 4	63 9	65 5	67 2
13 6	98 8	101 0	103 5	106 0	108 6	22 2	60 4	61 9	63 4	64 9	66 5
13 7	98 0	100 3	102 7	105 2	107 8	22 4	59 9	61 3	62 8	64 4	66 0
13 8	97 2	99 6	102 0	104 5	107 0	22 6	59 4	60 7	62 3	63 8	65 4
13 9	96 5	98 8	101 2	103 7	106 2	22 8	58 9	60 2	61 7	63 3	64 8
14 0	95 8	98 1	100 5	103 0	105 5	23 0	58 4	59 7	61 2	62 7	64 3
14 1	95 1	97 4	99 7	102 2	104 7	23 4	57 3	58 7	60 2	61 7	63 2
14 2	94 5	96 7	99 0	101 5	104 0	23 8	56 4	57 7	59 1	60 6	62 0
14 3	93 8	96 0	98 3	100 8	103 3	24 2	55 4	56 2	58 1	59 6	61 0

TABLE 36—DETERMINATION OF PER CENT HEMOGLOBIN—(Continued)

Colorimeter reading mm	Temperature of the standard											
	15 S°	16 S	17 S°	18 S	19 S	20 S	21 S	22 S	23 S	24 S	25 S	26 S
13 7	77 0	78 4	9 9	81 4	82 9	84 5	86 1	88 0	89 9	91 8	93 7	95 7
13 8	76 4	77 8	79 3	80 8	82 3	83 9	85 5	87 3	89 2	91 1	93 0	94 7
13 9	75 8	77 2	78 7	80 2	81 8	83 3	84 9	86 7	88 6	90 5	92 4	94 0
14 0	75 2	76 7	78 2	79 6	81 3	82 8	84 3	86 1	88 0	89 9	91 8	93 4
14 1	74 6	76 1	77 6	79 1	80 7	82 2	83 7	85 5	87 3	89 2	91 1	93 8
14 2	74 0	75 5	77 1	78 6	80 1	81 6	83 2	84 9	86 7	88 6	90 5	92 9
14 3	73 5	75 0	76 6	78 1	79 6	81 1	82 7	84 4	86 1	87 9	89 8	91 6
14 4	73 0	74 5	76 0	77 5	79 0	80 6	82 2	83 8	85 5	87 3	89 2	91 1
14 5	72 5	74 0	75 4	76 9	78 4	80 0	81 6	83 2	84 9	86 7	88 6	90 5
14 6	72 1	73 5	74 9	76 3	77 8	79 4	81 0	82 6	84 3	86 1	88 0	89 9
14 7	71 6	73 0	74 4	75 8	77 2	78 8	80 4	82 0	83 7	85 5	87 4	89 3
14 8	71 2	72 5	73 9	75 3	76 7	78 2	79 8	81 5	83 2	85 0	86 8	88 7
14 9	70 8	72 0	73 4	74 8	76 2	77 6	79 2	80 9	82 7	84 3	86 2	87 9
15 0	70 2	71 6	73 0	74 4	75 8	77 3	78 6	80 4	82 2	83 9	85 4	87 4
15 2	69 1	70 6	72 0	73 4	74 9	76 3	77 7	79 3	81 0	82 7	84 3	86 1
15 4	68 4	69 7	71 1	72 4	73 8	75 3	76 8	78 4	80 0	81 6	83 3	84 8
15 6	67 4	68 8	70 1	71 4	72 8	74 3	75 9	77 5	79 0	80 6	82 2	83 8
15 8	66 5	67 9	69 2	70 4	71 9	73 3	74 8	76 4	78 0	79 6	81 2	82 8
16 0	65 8	67 1	68 4	69 7	71 0	72 4	73 8	75 4	77 0	78 6	80 3	81 9
16 2	64 9	66 2	67 6	69 2	70 1	71 5	72 9	74 5	76 1	77 7	79 4	81 1
16 4	64 1	65 3	66 8	68 6	69 3	70 6	72 0	73 6	75 2	76 8	78 5	80 2
16 6	63 3	64 6	65 8	67 1	68 4	69 7	71 2	72 7	74 3	75 8	77 4	79 1
16 8	62 6	63 8	65 2	66 5	67 8	69 1	70 4	71 9	73 4	74 9	76 5	78 1
17 0	61 9	63 1	64 4	65 6	66 9	68 1	69 6	71 0	72 4	74 0	75 6	77 2
17 2	61 1	62 3	63 6	64 8	66 1	67 4	68 8	70 2	71 7	73 2	74 7	76 3
17 4	60 3	61 6	62 9	64 1	65 3	66 6	67 9	69 3	70 8	72 3	73 8	75 3
17 6	59 6	60 9	62 2	63 4	64 6	65 8	67 0	68 5	70 0	71 5	73 0	74 5
17 8	59 0	60 3	61 4	62 7	63 9	65 1	66 4	67 8	69 1	70 7	72 3	73 8
18 0	58 5	59 6	60 8	62 0	63 2	64 5	65 8	67 0	68 3	69 8	71 4	73 1
18 2	57 9	59 0	60 2	61 3	62 5	63 8	65 0	66 3	67 7	69 1	70 6	72 3
18 4	57 2	58 3	59 6	60 7	61 9	63 1	64 3	65 7	67 1	68 5	69 9	71 4
18 6	56 6	57 7	59 0	60 1	61 2	62 3	63 5	64 9	66 3	67 7	69 2	70 6
18 8	55 9	57 0	58 3	59 4	60 6	61 7	62 9	64 1	65 5	66 9	68 4	69 9
19 0	55 3	56 4	57 7	58 8	59 8	61 0	62 0	63 3	64 7	66 2	67 7	69 2
19 2	54 7	55 8	57 1	58 1	59 2	60 2	61 3	62 6	64 0	65 5	67 0	68 4
19 4	54 1	55 2	56 3	57 4	58 5	59 7	60 9	62 2	63 6	64 9	66 2	67 7
19 6	53 6	54 6	55 7	56 8	57 9	59 1	60 3	61 6	62 8	64 3	65 5	66 9
19 8	53 0	54 1	55 2	56 3	57 4	58 5	59 7	60 6	62 1	63 4	64 8	66 2
20 0	52 5	53 6	54 6	55 7	56 8	57 9	59 1	60 2	61 6	62 8	64 1	65 6
20 4	51 5	52 5	53 6	54 6	55 6	56 7	57 9	59 0	60 2	61 5	62 7	64 3

opposite the average mm reading,¹ interpolating when necessary. Convert the per cent figure into grams per 100 cc of blood by using Table 43 (p 489).

An error of over 2 per cent is unusual in routine work and, with research care, it can be held within 1 per cent.

Instead of being heated the acid hematin solutions may stand at room temperature 24 hours, or even several days before being used.

¹ If the colorimeter reading is higher than 20 4 mm cent in the table and then divide by 2. A better of acid hematin diluting to 50 or 25 cc instead of divided by 2 or 4.

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TABLE 37—HEMOGLOBIN TABLE FOR HIGHER TEMPERATURES

Colorimeter reading mm	Temperature of the standard										
	27 5	28 5	29 5	30 5	31 5	mm	27 5	28 5	29 5	30 5	31 5
9 4	142 9	146 2	149 6	153 0	156 0	14 4	93 2	95 4	97 6	100 1	102 6
9 5	141 5	144 7	148 0	151 1	154 3	14 5	92 6	94 8	97 0	99 5	101 9
9 6	140 1	143 3	146 5	149 7	152 9	14 6	92 0	94 1	96 3	98 8	101 2
9 7	138 7	141 8	145 0	148 4	151 8	14 7	91 4	93 5	95 6	98 1	100 5
9 8	137 1	140 3	143 5	147 1	150 6	14 8	90 8	92 9	95 0	97 4	99 8
9 9	135 6	138 8	142 1	145 6	149 1	14 9	90 2	92 3	94 4	96 7	99 2
10 0	134 2	137 3	140 5	144 1	147 7	15 0	89 6	91 7	93 8	96 1	98 5
10 1	132 8	135 9	139 0	142 6	146 2	15 2	88 4	90 5	92 6	94 9	97 3
10 2	131 6	134 6	137 7	141 3	144 8	15 4	87 2	89 3	91 4	93 7	96 0
10 3	130 4	133 4	136 5	140 0	143 4	15 6	86 0	88 1	90 2	92 6	94 7
10 4	129 1	132 1	135 2	138 6	142 0	15 8	85 0	87 0	89 1	91 2	93 5
10 5	127 8	130 8	133 9	137 4	140 8	16 0	84 0	85 9	87 9	90 1	92 4
10 6	126 6	129 6	132 7	136 0	139 6	16 2	82 9	84 8	86 6	89 0	91 2
10 7	125 4	128 4	131 5	134 7	138 0	16 4	81 8	83 8	85 8	88 0	90 1
10 8	124 2	127 1	130 2	133 4	136 6	16 6	80 9	82 8	84 8	86 9	89 0
10 9	123 1	126 0	129 0	132 2	135 4	16 8	80 0	81 9	83 8	85 8	87 9
11 0	122 0	124 9	127 9	131 1	134 3	17 0	79 1	81 0	82 8	84 8	86 9
11 1	120 9	123 8	126 8	129 9	133 1	17 2	78 2	80 0	81 8	83 8	85 9
11 2	119 9	122 7	125 6	128 8	132 0	17 4	77 3	79 1	80 9	82 9	84 9
11 3	118 9	121 5	124 5	127 7	130 9	17 6	76 4	78 2	79 9	81 9	83 9
11 4	117 8	120 4	123 4	126 6	129 7	17 8	75 5	77 3	79 0	81 0	83 0
11 5	116 8	119 4	122 4	125 5	128 6	18 0	74 6	76 4	78 2	80 1	82 1
11 6	115 7	118 4	121 4	124 4	127 5	18 2	73 8	75 5	77 3	79 2	81 2
11 7	114 7	117 4	120 4	123 4	126 4	18 4	73 0	74 7	76 5	78 4	80 4
11 8	113 7	116 4	119 3	122 3	125 3	18 6	72 2	73 9	75 7	77 6	79 5
11 9	112 8	115 5	118 2	121 1	124 1	18 8	71 4	73 1	74 9	76 8	78 6
12 0	111 8	114 5	117 2	120 1	123 0	19 0	70 7	72 3	74 0	75 9	77 8
12 1	110 9	113 6	116 3	119 2	122 1	19 2	70 0	71 6	73 2	75 0	76 9
12 2	110 0	112 7	115 4	118 3	121 2	19 4	69 2	70 8	72 4	74 2	76 1
12 3	109 1	111 8	114 5	117 3	120 1	19 6	68 5	70 1	71 7	73 5	75 4
12 4	108 3	110 8	113 4	116 2	119 0	19 8	67 8	69 4	71 0	72 8	74 7
12 5	107 4	109 9	112 4	115 2	118 0	20 0	67 1	68 7	70 3	72 1	73 9
12 6	106 5	109 0	111 5	114 3	117 1	20 2	66 4	67 9	69 5	71 3	73 1
12 7	105 6	108 1	110 7	113 5	116 2	20 4	65 8	67 3	68 8	70 7	72 4
12 8	104 8	107 3	109 9	112 7	115 4	20 6	65 2	66 7	68 3	70 0	71 9
12 9	104 0	106 5	109 0	111 7	114 4	20 8	64 5	66 1	67 7	69 4	71 4
13 0	103 3	105 6	108 2	110 8	113 5	21 0	63 9	65 4	67 0	68 7	70 4
13 1	102 5	104 9	107 3	110 0	112 7	21 2	63 3	64 6	66 3	68 0	69 8
13 2	101 7	104 1	106 5	109 2	111 9	21 4	62 7	64 2	65 7	67 3	69 0
13 3	100 9	103 4	105 7	108 4	111 1	21 6	62 1	63 5	65 1	66 7	68 3
13 4	100 2	102 6	105 0	107 6	110 3	21 8	61 5	63 0	64 5	66 2	67 7
13 5	99 6	101 9	104 2	106 8	109 4	22 0	61 0	62 4	63 9	65 5	67 2
13 6	98 8	101 0	103 5	106 0	108 6	22 2	60 4	61 9	63 4	64 9	66 5
13 7	98 0	100 3	102 7	105 2	107 8	22 4	59 9	61 3	62 8	64 4	66 0
13 8	97 2	99 6	102 0	104 5	107 0	22 6	59 4	60 7	62 3	63 8	65 4
13 9	96 5	98 8	101 2	103 7	106 2	22 8	58 9	60 2	61 7	63 3	64 8
14 0	95 8	98 1	100 5	103 0	105 5	23 0	58 4	59 7	61 2	62 7	64 3
14 1	95 1	97 4	99 7	102 2	104 7	23 4	57 3	58 7	60 2	61 7	63 2
14 2	94 5	96 7	99 0	101 5	104 0	23 8	56 4	57 7	59 1	60 6	62 0
14 3	93 8	96 0	98 3	100 8	103 3	24 2	55 4	56 2	58 1	59 6	61 0

They must be shaken well before filling the colorimeter cup since acid hematin "solution" is a suspension

The modified method, (C) below, is recommended for routine use, as it requires less time and there is less chance of contaminating the permanent standard

Preservation of the standard Keep it in a Pyrex flask, corked tightly with a *rubber stopper from which the powdered material has been removed* by boiling in dilute sodium hydroxide and vigorous scrubbing. The standard, after being used, is stored in a second flask. Before the main stock is exhausted, check used standard against it by making estimations of the same acid hematin preparation with each. If they agree, pour the used standard into the other flask and use it again. If the used standard has become contaminated, it will usually be off color as well as give incorrect readings. If it seems to be incorrect, filter it. Recheck the setting of the colorimeter, and then check the filtrate before deciding to discard it. With reasonable care, no change in the solution occurs during months or years of repeated use.

(c) *Modified Osgood Haskins Method*¹—This is recommended for laboratories making more than 10 hemoglobin estimations per day. It involves the use of acid hematin as a standard but since it is used for only one week, there is no detectable change in its value.

(1) **Technic** Make a *careful* estimation by the original Osgood Haskins method (p 463) on the first blood obtained. Heat to develop the full color and keep the entire 100 cc sample of acid hematin in a brown bottle labelled with the per cent of hemoglobin found, the temperature of the permanent standard, and the colorimeter reading corresponding to it (e.g., 111.5 per cent at 11.5 mm and 25.5° C.)²

For the rest of the week use this as the standard, shake to secure uniform suspension before using, place it in the standard cup and set at the same reading as that noted on the bottle (e.g., 11.5 mm). The temperature of the standard is not taken, since the color of the acid hematin does not vary.

Calculation Use the same table as for the original method, finding the per cent hemoglobin opposite the mm reading in the same temperature column as that marked on the bottle containing the acid hematin standard.

¹ Osgood E. E. Haskins H. D. and Trotman F. E. A Simplification of the Osgood Haskins Hemoglobin Method. J. Lab. & Clin. Med. 16: 482-486 (Feb.) 1931.

² If the reading obtained involves interpolation e.g. 100.5 per cent at 10.55 mm and 16° find the same per cent figure elsewhere in the table e.g. 100.5 per cent at 11.5 mm and 20.5° and note these figures on the label on the bottle.

By this modified method much time is saved and one lot of 50 cc of permanent standard can be kept in good condition for a year or more. Twenty-five estimations can be made in 50 minutes or less.

The maximum error in routine work is not over 4 per cent.

(d) *Van Slyke's Gasometric Methods*—These are the most accurate of all methods now in use. They are of value for research work and for checking other methods, but are unsatisfactory for clinical laboratories because the technic is very exacting and too much time is required for an estimation. The principle of the older oxygen capacity method¹ is the measurement of the amount of oxygen liberated when fully oxygenated hemoglobin is changed to methemoglobin by the action of potassium ferricyanide and is exposed to a partial vacuum. It has been found that 18.5 cc of dry oxygen measured at 0° C. and 760 mm of mercury will be liberated if 100 cc of the blood contains 13.8 grams of hemoglobin.

The newer carbon monoxide method,² using the manometric apparatus, is the most accurate. The principle involved is the measurement of the amount of carbon monoxide liberated from blood saturated with the gas when it is exposed to the action of an acidified ferricyanide reagent and a partial vacuum. It has been determined that hemoglobin combines with equivalent quantities of carbon monoxide and oxygen.

(e) *The Acid Hematin Method of Cohen and Smith*—This involves the use of a colorimeter and the preparation (about once a month) of a standard solution of acid hematin. The latter is made by first estimating hemoglobin in a sample of fresh blood very carefully by Van Slyke's method and then preparing from the blood (by dilution and treatment with hydrochloric acid) a rather concentrated acid hematin solution of exact strength. This is kept in the icebox. A more dilute solution is prepared every few days for use in the colorimeter. The method is very good but requires an expert to make the standard solution. It is quite suitable for research work, if the investigator is competent to secure accurate Van Slyke estimations.

(f) *The Original Sahli Method*—This is an acid hematin method, using a comparator for color matching and an acid hematin standard. The ordinary type of Sahli apparatus is worthless because the acid hematin used as the standard fades too rapidly. Other color standards have been used by manufacturers but not even the colored glass standards have proved satisfactory. A reading of 100 on the old Sahli was supposed to indicate a hemoglobin content of 17.3 grams per 100 cc. The glass standards have varied hemoglobin equivalents and some of them correspond to a different hemoglobin content from that claimed for them by the manufacturer.

When a correct standard solution is used in the Sahli the estimations are surprisingly accurate under certain conditions. These conditions are the inside diameter of the graduated tube and of the tube containing the standard solution must be the same and the Sahli pipette and graduated tube must be accurately marked. These specifications can be met without unreasonable extra expense.

¹ Van Slyke, D. D. and Stadie, W. C. The Determination of the Gases of the Blood. *J Biol Chem* 40: 1-42, 1921.

² Van Slyke, D. D. and Hiller, Alma. Gasometric Determination of Hemoglobin by the Carbon Monoxide Capacity Method. *J Biol Chem* 78: 807-819 (Aug.) 1923.

(g) *The Tallqvist Method*—This is very easy. Simply place a drop of blood on the white blotting paper furnished and, as soon as the gloss is off, match it by reflected light with the lithographed scale, 100 per cent indicates 13.8 grams of hemoglobin per 100 cc of blood. It is extremely inaccurate, errors of 30 per cent being common.

(h) *The Dare Method*—A thin film of blood is drawn by capillarity between the ground glass and clear glass plates and compared by means of transmitted yellow light with a circular wedge of red glass which is rotated till it matches the blood film. The percentage of hemoglobin is read off directly, 100 per cent is supposed to correspond to 16 gm in the newer instruments and to 13.8 grams of hemoglobin per 100 cc of blood in the older models.

The readings secured are often very inaccurate, errors of 30 per cent are not rare, and, considering the expense of the apparatus, the method is not preferable to the Tallqvist.

(i) *The Newcomer Method*—In the Newcomer method a disc of yellow glass is placed on one side of the colorimeter or is built into a colorimeter and is matched with an acid hematin solution. The method is more accurate than the Dare and Tallqvist but the color is so pale that a match is difficult and deviations of plus or minus 12 per cent may be obtained from the average for the particular disc and some of the discs differ significantly from the value of 16.92 gm per 100 cc which is supposed to correspond to 100 per cent in this method. Therefore new discs should be checked by a series of estimations on oxalated blood, the hemoglobin values of which have been determined by the Van Slyke or Osgood Haskins method.

(j) *A Method Using the Photometer*¹—This method has undergone rapid development and further improvements are to be expected. It is worthy of investigation by those interested in methods of research accuracy or in charge of very large laboratories, but it offers few advantages over methods (b) and (c) for these purposes.

Any photoelectric colorimeter may be used for hemoglobin estimations preparing the acid hematin as directed for the Osgood Haskins method and calibrating a curve on semilogarithmic paper by use of acid hematin solutions, the hemoglobin equivalent of which has been determined by the Osgood Haskins method.

(k) *The Haden Hauser Method*—In this method an acid hematin solution prepared in the white cell diluting pipette is compared with a wedge shaped glass standard. It is convenient and more accurate than the Dare and Tallqvist methods but has the errors inherent in all acid hematin methods in which the full color is not developed by heat or by standing at least 24 hours before reading and the additional errors that are built into the white cell diluting pipettes. Even the Bureau of Standards allows a considerable error in pipettes bearing its certificate.

(l) *Blood Iron Method*²—A number of methods have been suggested for the determination of blood iron. This is actually a determination of the hemoglobin,³ because hemoglobin contains 0.335 per cent of iron and there is only about 0.5 mg

¹ Sanford A. H. Sheard C. and Osterberg A. E. The Photometer and Its Use in the Clinical Laboratory. *Am J Clin Path* 3: 405-420 (Nov.) 1933.

² Kennedy R. R. Quantitative Determination of Iron in Tissues. *J Biol Chem* 74: 385 (Aug.) 1927.

Wong S. Y. Colorimetric Determination of Iron and Hemoglobin in Blood. *J Biol Chem* 77: 409 (May) 1928.

³ Myers V. C. and Eddy Helen M. The Hemoglobin Content of Human Blood. *J Lab & Clin Med* 24: 502-511 (Feb.) 1939.

of iron present in 100 cc of plasma. The method is almost as accurate as the Van Slyke and Osgood Haskins methods. It is easier to do than the oxygen capacity or carbon monoxide combining power but considerably more difficult than the Osgood Haskins method. Hence it is easier to determine hemoglobin by the Osgood Haskins method and multiply the result in grams by 3.35 to get the blood iron in mg per 100 cc. The principle involved in the blood iron method is to digest the hemoglobin with perchloric¹ or persulphuric acid, oxidize the iron to the ferric form, produce the reddish ferric thiocyanate and compare with a standard in the colorimeter. Detailed directions are given in the references.

6 Red Cell Counting—Oxalated venous blood is recommended for all cell counting because it eliminates most of the sources of error involved in the use of capillary blood, it permits the checking of results

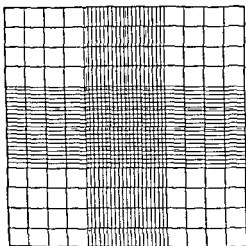


FIG. 21—Neubauer ruling

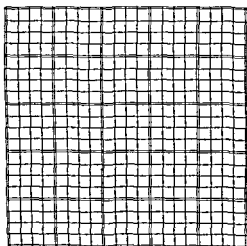


FIG. 22—Improved Neubauer ruling (central area only)

on identical portions, and it gives results on the same blood specimen as that used for hemoglobin, and cell volume determination.

It is desirable that the counting chamber and pipettes be tested by the Bureau of Standards.

(a) *Technic*—The slide and pipettes must be clean and dry before being used. There must be no trace of grease or lint on the counting chamber or the cover glass. These are to be washed with soap and luke warm water, then dried with a clean soft cloth that is free of lint, a freshly laundered old linen handkerchief is ideal. Place the cover glass in position over the ruled area and a little back from the edge of the slide. Inspect the pipettes to see that the capillary tube contains no dirt or old blood and that they are dry enough so that the glass ball in the bulb will roll freely. Fill a small watch glass with the diluting fluid to be used. Have everything in readiness before drawing the blood if capillary blood is used.

¹ Should not be used because of danger of explosion.

If using oxalated blood, hold the tube almost horizontal and vigorously tap the bottom with the fingers for at least 30 seconds to mix the blood completely, then tip the tube so that blood runs almost to the mouth, insert the tip of the pipette and draw blood up to or just beyond the 0.5, or if a low count is expected to the 1.0, mark. See Table 8 for the errors that result if only a small number of cells are counted.

If using capillary blood, clean the skin where the puncture is to be made with alcohol, using the tip of the finger or the lobe of the ear in adults, in small children the toe or heel. When the skin is *dry*, make a quick stab with the lancet, deep enough to draw 4 or 5 drops of blood without pressure. Wipe off the first 3 drops and as soon as the next drop of blood collects, draw it quickly into the pipette exactly to or slightly beyond the desired mark. Hold the pipette horizontally, wipe the excess blood off the tip with a clean, dry towel and adjust the blood in the capillary to the mark by stroking the tip with the finger or with some non absorbent surface such as thin rubber. Since absorbent material takes up the plasma more rapidly, the cells are concentrated when such material is used to draw the blood down to the desired mark. This error in technic may result in counts as much as 500,000 too high. After the blood column is adjusted, carefully wipe the outside of the tip to remove any material adhering to it. When using capillary blood, work speedily so as to get the blood diluted before clotting begins.

Hayem's solution should not be used since it tends to give low counts.

Draw in Toisson's diluting fluid with the pipette in an almost horizontal position, at the same time mixing the blood with the fluid by semi rotations of the pipette, rolling it between the thumb and finger. When the liquid is near the top of the bulb, raise the pipette to a vertical position allowing the air to pass out of the bulb and slowly fill to the mark. Keep the pipette in a horizontal position until the blood is uniformly mixed. Close the tip with the finger, kink the rubber tubing over the end of the pipette or remove it entirely and hold it with the thumb. Shake¹ it for two minutes, but do not shake in the direction of the long axis of the pipette, also do not revolve the contents in one direction only. Shaking the tube in the long axis may, by centrifugal force, throw cells into the capillary tube and thus remove them from the diluted portion. Revolving the pipette in one direc-

¹ A satisfactory mechanical shaker is obtainable from the Braun Knecht Hermann Co. San Francisco or from the National Appliance Co. Portland Ore.

tion only may also concentrate the cells in the outer portion of the mixture

While the blood is still well mixed, make the mount. Blow out and discard at least 2 drops. Wipe off the tip with absorbent material, blow out 2 more drops, then wipe off the tip with non absorbent material, and then touch the tip of the pipette to the central platform of the slide at the edge of the cover glass and let the space fill by capillarity. When it has flowed three fourths of the way across, withdraw the pipette because enough liquid will be left to fill the space. If air bubbles are present, clean the slide and cover glass more carefully, then make another mount after shaking. If liquid has overflowed into the grooves of the slide, make another mount. The fluid should flow smoothly across the chamber without any jerks or stops. Its flow may be regulated with a finger over the upper end of the pipette as in using ordinary chemical pipettes.

Place the slide on the stage of the microscope immediately and locate the ruled area. Allow 3 minutes for settling of the cells, then examine with the low power or, better, the 8 mm objective for complete settling and uniformity of distribution, if the cells are unevenly distributed, make another mount after thorough shaking of the diluted blood. Lower the condenser a little, reduce the light by means of the diaphragm until the rulings and the red cells are seen most distinctly. An 8 mm objective is best for counting red cells, but the 4 mm (high power) may be used. Never use the low power because the cells appear so small and close together that omissions and duplications are almost certain.

Count¹ 5 groups of 16 small squares. Most use the diagonal squares from the upper left hand to the lower right hand corners, but the four corners and one of the center squares are equally satisfactory. If the count is less than 300, count enough more squares to bring the total above this figure or, better, count another mount and average the totals. If an uneven distribution is suspected, keep the totals of each group of 16 squares separate. These should not differ by more than 20 cells. The error depends more on the total number of cells counted than on the area (see page 172).

Rule for cells lying on boundary lines. For each individual square, count only those cells in contact with the left hand and upper boundary lines, but not those touching the right hand and lower lines. In the improved Neubauer ruling (Levy) there is a double line bordering

¹ Use of a hand tally such as a Veedor counter is a great convenience in large laboratories

each block of 16 small squares. The actual boundary is not the inner most of the two lines but a shady line which can be seen by proper focusing midway between the two engraved lines, count only those cells in contact with this mid line.

Calculation

Blood taken	Red blood cells per cubic millimeter
0.5	Cells per small square $\times 800,000$ or cells per 100 squares $\times 8000$ Average total cells per 80 small squares $\times 10,000$
1.0	Cells per small square $\times 400,000$ or cells per 100 squares $\times 4000$ Average total cells per 40 small squares $\times 10,000$

Each small square has an area of one four hundredth square mm, and the liquid has a depth of 0.1 mm. Therefore, one four thousandth of a cubic millimeter of diluted blood lies above the small square but, in terms of undiluted blood, the volume is $1/400,000$ if a 1 in 100 dilution is made by drawing blood up to 10 mark or $1/800,000$ of a cubic millimeter if a 1 in 200 dilution is made by drawing blood up to 0.5 mark.

(b) *Apparatus*—(1) *Counting chamber*, preferably the Levy Neubauer ruling with Bureau of Standards certificate. The older type which has the ruled area on a separate piece of glass cemented to the slide is perfectly good but must be handled more carefully. Organic solvents such as alcohol or ether must not come in contact with it, and the slide must not be exposed to heat or sunlight.

The counting chamber should be wiped clean and dry as soon as the count is finished. If a clean dry cloth is used and care is taken not to touch the ruled area with the fingers or any greasy object, it is not necessary to wash the slide each time.

(2) *Cover slip*. The heavy type especially designed for blood counting chambers is the one to use. The Bureau of Standards certified cover slips are most satisfactory because they are perfectly ground and thus give a uniform depth to the counting chamber.

(3) *Pipettes*. The Thoma type with Bureau of Standards certificate is recommended. The Trenner automatic type is a great convenience since the capillary fills with exactly the right amount of blood thus eliminating the necessity of adjusting it to the mark but these pipettes are likely to break at the point where the bulb is fused on to the capillary. The chief objection for use with capillary blood is the rather large amount of blood required. A smaller size is being constructed to obviate this.

To clean the pipettes, first draw water through them preferably with a suction pump. If blood is clotted in the capillary, dislodge it with a horse hair or stiff fine suture material. In some cases to remove the blood completely, it may be necessary to draw in 10 per cent sodium hydroxide, let it stand a while and then draw through it water dilute acid, and more water. Water is removed by successive use of 95 per cent alcohol and ether. A single dehydrating agent may be used

if pure acetone or C P anhydrous methyl alcohol is available but these are expensive for the purpose. Remove the vapor completely.

(c) *Reagents*—(1) *Diluting fluid* Toisson's solution is recommended for the following reasons. Its specific gravity and viscosity are such that the cells are kept in suspension for several minutes, thus allowing plenty of time for making the mount, without danger of altering the cell distribution. The stain which it contains colors the white cells so that they are not included in the red cell count. When the white cell count is high, especially in association with anemia, the inclusion of the white cells with the red cells during the count may produce gross errors. In leukemias the white cell count may be made in the same mount as the red cells by counting the stained cells in 100 squares and using the same factor for calculation as for the red cells.

The solution used for diluting is made up fresh each week or two from concentrated stock solutions as follows: 5 parts of concentrated Toisson's solution, 10 parts of distilled water, and 1 part of 0.2 per cent alcoholic methyl violet. Any precipitate that forms on standing should be filtered off.

Concentrated Toisson's solution Dissolve 45 grams of sodium sulphate crystals and 5 grams of sodium chloride in 160 cc of water; filter, add to the filtrate 170 cc of C P glycerol and dilute to 350 cc. This solution remains perfectly clear and free of molds indefinitely.

7 **White Cell Counting**—(a) *Technic*—Every step and precaution used in the red cell count applies equally to the white cell count with the exceptions that a different pipette and diluting fluid are used. Very high white counts, such as occur in some leukemias, may be counted as described above under the red cell count, or the blood may be diluted with the white cell diluting fluid in the red cell pipette. The usual method of enumeration may be used for the high counts but the red cell ruling (central portion of the slide) is preferable, especially when the white cell pipette is used for dilution.

Count the dark dots representing the stained nuclei of the white cells; the cytoplasm of the white cells becomes transparent and the red cells are destroyed by the acid in the diluting fluid. It is customary to count the four large squares, each 1 square mm in area and containing 16 medium sized squares, at the corners of the ruled area (Fig. 21). Uniformity of distribution is not expected. The 8 mm objective is preferable for counting, but the low power may be used if the count is not too high. If there is any doubt about the identification of the dark dots, use the higher power; the nuclei have a somewhat granular appearance at a certain focus and in the lobocytes (polymorphonuclears) are lobulated.

Calculation The large square has an area of 1 square mm, the depth is 0.1 mm, the dilution is 1 in 10 or 1 in 20, therefore, the liquid over each square contains $\frac{1}{100}$ or $\frac{1}{200}$ cubic millimeter of the original

blood See the table below for the factors to use in the calculation
The accuracy depends on the total number of cells counted

Blood taken	White blood cells per cubic millimeter
o 5	Average number of cells per large square $\times 200$
1 o	Average number of cells per large square $\times 100$
o 5 in red cell pipette	Average number of cells per large square $\times 2000$
1 o in red cell pipette	Average number of cells per large square $\times 1000$

(b) *Reagents* —(1) Diluting fluid (3 per cent acetic acid) Dilute 15 cc. of 10 per cent acetic acid with 35 cc. of distilled water and add 1 drop of 1 per cent aqueous methylene blue Prepare a fresh solution each week

8 The Preparation and Study of Stained Smears —The differential white cell count is made by spreading blood in a thin film on a clean slide, fixing it and staining with a good differential stain, such as Wright's modification of the Romanowsky stain The slide is then examined under an oil immersion lens and the white cells classified

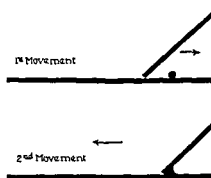


FIG 23

as seen until 200 have been counted

(a) *The Essentials for Making a Good Smear Are* —(1) A clean unscratched slide If the slide is new, a thorough washing with soap and water, rinsing well in water before drying and polishing with a cloth may be sufficient Old slides should be washed first in alkali, then in acid, rinsed thoroughly, and finally dried with a soft cloth If

grease spots are not removed by this technic, use alcohol

(2) A small drop of unclotted, well mixed blood should be placed far enough from one end of the slide to allow room for a label White marking ink is now available which may be used instead of a label or the slide may be labelled with a carborundum pencil if it is to be kept permanently The size of the drop should be such that the smear extends almost, but not quite, to the end of the slide

(3) The slide should be laid on a firm surface, such as a table top, a second slide brought in contact with it at an angle of 60° and pulled back until it touches the drop, then pushed across the slide, drawing the blood behind it with an even, steady, moderate pressure (Fig 23) Slow movement produces a thin smear, rapid, a thick one, thus one may regulate the thickness by the rate of movement of the smearing slide

(4) As soon as the slide is smeared, it should be allowed to dry completely in the air (not heated or waved in the air)

(b) *The Criteria for Judging a Good Smear Are* —(1) It should have a smooth even appearance free from "holes" "Holes" indicate grease spots on the slide and show that the slides have not been thoroughly cleaned Marrow contains fat so that such holes cannot be avoided in making smears of marrow obtained by sternal puncture Transverse and longitudinal streaks, or a wavy appearance are due to a jerky uneven motion of the smearing slide This is overcome by putting the slide on the table to smear instead of trying to hold it in the hand

(2) The smear should not extend to either end of the slide Avoid this by using a smaller drop of blood

(3) The smear should not be too thick Under the microscope, at least at the thin end of the smear, the red cells should not touch each other but be evenly spread If too thick it will not dry rapidly enough and the red cells will be clumped together and crenated and many lobocytes (polymorphonuclears) will appear similar to small lymphocytes Avoid this by moving the smearing slide more slowly

(4) The nucleated cells should be distributed evenly throughout the smear and not concentrated at the ends or edges Avoid this by holding the smearing slide at an angle of 60° or more from the horizontal

(c) *The Essentials for Good Staining Are* —(1) The use of a good stain Wright's modification of the Romanowsky stain is probably best It is purchased as small tablets from various manufacturers These are ground up and 10 cc of absolute anhydrous methyl alcohol is added for each tablet (0.050 gram) used The mixture is shaken and allowed to stand 24 hours or longer and it should then be filtered before use, as not all of the stain dissolves Fresh stain should be made up frequently, at least once a month preferably oftener, and filtered as often as precipitate forms Laboratories using large quantities of Wright's stain may to advantage use dry powdered stain (made by the National Aniline and Chemical Co.), dissolving 170 mg in 100 cc of C P anhydrous methyl alcohol and let it stand 24 hours with occasional shaking then filter

(2) The stain must be well corked to prevent evaporation of alcohol and taking up of moisture

(3) The slide,¹ held horizontally, is well covered with stain and allowed to stand for 1 minute During this period the methyl alcohol in the stain fixes the cells

¹ A wax pencil mark transversely across the slide about one half inch from the end at which the smear was started will hold the stain back and leave a clean place for holding the slide.

(4) Without pouring off the stain a sufficient *number of drops* of McJunkin's¹ buffer phosphate pH 6.4 is added and mixed by tilting the slide back and forth to produce a glossy sheen on the surface. The buffer phosphate solution is a mixture of 73 cc of M/15 monopotassium phosphate solution plus 27 cc of M/15 disodium phosphate solution.² In an emergency, distilled water may be used instead of the buffer phosphate, but is not nearly so satisfactory. Leave the phosphate stain mixture on the slide for the time period determined by trial, which gives the best results with the particular stain used. This is usually about 4 minutes, but varies from 45 seconds to 15 minutes according to the age and quality of the stain. Old stain requires longer and the Wright's stain now available requires a much longer staining period than the stains which were available 10 or 15 years ago.

TABLE 38—IDENTIFICATION OF CELLS CONTAINING NEUTROPHIL GRANULES*

Nucleoli	Nucleus	Name of cell	Number†
Present	Round or oval	Neutrophil progranulocyte S (Neutrophil promyelocyte I)	68
Absent	Round or oval	Neutrophil granulocyte (Neutrophil myelocyte)	70-73
	Bean or kidney shaped	Neutrophil metagranulocyte (Neutrophil metamyelocyte)	74-75
	Curved rod	Neutrophil rhabdocyte (Neutrophil staff cell)	76-79
	Lobed or segmented	Neutrophil lobocyte (Polymorphonuclear)	80-85

* If the granules are scarce, big and blue or the cytoplasm contains vacuoles or is bluer than normal, they are toxic neutrophils (87-92) but are classified otherwise as in the table.

† The numbers refer to the illustrations of the cells in Osgood, E. E. and Ashworth, Clarence M. *Atlas of Hematology*, Pp. 255. J. W. Stacey, Inc., San Francisco, 1937, from which this table is reproduced by permission of the copyright owners.

(5) As soon as the staining time is up, do not pour off the stain but, holding the slide level, float it off with running water and wash thoroughly with running tap water for at least 30 seconds. Wipe the back of the slide with a piece of paper towel.

¹ McJunkin, F. A. A Benzidine Polychrome Stain for Blood. *J. A. M. A.*, 74: 17 (January 3) 1920.

² The buffer may be prepared by dissolving 6.63 grams of Merck's monopotassium phosphate and 2.56 grams of Merck's anhydrous disodium phosphate in 1 liter of distilled water. Add about 1 cc of chloroform as a preservative.

(6) The slide may then be blotted carefully with smooth filter paper, but it is better to stand it on end on a paper towel, leaning against the wall, until it is air-dried

(d) *The Criteria for Judging a Good Stain Are*—(1) If, on washing, the film fails to stick to the slide, it is a sign of poor fixation, due either to too thick a smear, to too short a time of fixation, or to too much moisture having been taken up by the stain from letting the bottle stand uncorked

TABLE 39—IDENTIFICATION OF CELLS CONTAINING EOSINOPHIL GRANULES

Nucleoli	Nucleus	Name of cell	Number*
Present	Round or oval	Eosinophil progranulocyte S (Eosinophil promyelocyte I)	93-94
Absent	Round or oval	Eosinophil granulocyte (Eosinophil myelocyte)	95-96
	Bean or kidney shaped	Eosinophil metagranulocyte (Eosinophil metamyelocyte)	97-99
	Curved rod	Eosinophil rhabdocyte (Eosinophil staff cell)	100-102
	Lobed or segmented	Eosinophil lobocyte (Eosinophil polymorphonuclear)	103-105

* The numbers refer to the illustrations of the cells in Osgood E E and Ashworth Clarence M Atlas of Hematology Pp 255 J W Stacey Inc San Francisco 1937 from which this table is reproduced by permission of the copyright owners

TABLE 40—IDENTIFICATION OF CELLS CONTAINING BASOPHIL GRANULES

Nucleoli	Nucleus	Name of cell	Number
Present	Round or oval	Basophil progranulocyte S (Basophil promyelocyte I)	107-108
Absent	Round or oval	Basophil granulocyte (Basophil myelocyte)	109-110
	Bean or kidney-shaped	Basophil metagranulocyte (Basophil metamyelocyte)	111
	Curved rod	Basophil rhabdocyte (Basophil staff cell)	112-113
	Lobed or segmented	Basophil lobocyte (Basophil polymorphonuclear)	114-115

The numbers refer to the illustrations of the cells in Osgood E E and Ashworth Clarence M Atlas of Hematology Pp 255 J W Stacey Inc San Francisco 1937 from which this table is reproduced by permission of the copyright owners

LABORATORY DIAGNOSIS

TABLE 41.—IDENTIFICATION OF CELLS CONTAINING AZUROPHIL GRANULES

Diameter of cell in relation to lobocyte Same or smaller	Size of granules	Nucleoli Present or absent	Chromatin structure	Shape of nucleus	Peroxidase stain	Name of cell	Number*
Larger	Coarse	Present or absent	Coarse	Round or oval, sometimes irregular or cloverleaf	Negative	Lymphocyte	17-19 22-24
				Round or oval, rarely irregular or cloverleaf	Negative	Prolymphocyte	10-11 13-15
				Round or oval rarely	Positive	Progranulocyte A (promyelocyte II)	60-66
				Round or oval horseshoe	Negative	Plasmacyte†	130
				Round or oval	Negative	Lymphoblast or granuloblast	4-5, 56
	Fine, diffuse	Present	Fine	Round or oval rarely horseshoe†	Negative	Monoblast	31 33-35
				Round or oval	Negative	Promonocyte	37-40
				Horseshoe or irregular	Positive or negative	Monocyte	42-51
				Horseshoe or irregular	Positive or negative	Megalokaryocyte	310-311
				Horseshoe or irregular	Negative		
3 times as large	Fine diffuse	Absent	Coarse clumps and strands	Coarse clumps and strands			
				Coarse clumps and strands			

Pp 255 J W Stacey Inc San Francisco

M: Atlas of Hematology

* The numbers refer to the illustrations of the cells in Osgood, B. E. and Ashworth, Clarence M.

† Rieder cell

‡ Cytoplasm opaque

§ Plasma cells with azurophilic granules are a very rare

TABLE 42—IDENTIFICATION OF CELLS CONTAINING NO GRANULES

Cytoplasm	Nucleoli	Chromatin structure	Diameter of nucleus in relation to diameter of cell	Size of cell in relation to neutrophil lobocyte	Peroxi- dase stain	Name of cell	Numbers
Opaque	Absent	Pycnotic	Less than half	Smaller	Negative	Metakaryocyte†‡ (Normoblast)	155-162
		Coarse	Less than two-thirds	Smaller	Negative	karyocyte†‡ (Pronormoblast)	146-154
			More than two-thirds	Same or larger	Negative	Prokaryocyte† (Erythroblast)	139-143
			Less than half	Usually larger	Negative	Plasmacyte†	125-129
	Present	Coarse	Less than half	Usually larger	Negative	Proplasmacyte	119-122 124
		Fine	Less than two-thirds	Usually larger	Negative	Plasmablast	118
			More than two-thirds	Same or larger	Negative	Karyoblast† (Megaloblast)	132 134 137
			Present	Fine	More than half	Usually larger	Negative
Trans- parent	Present or absent	Coarse	More than half	Larger	Positive	Progranulocyte A (Promyelocyte II)	59
					Negative	Prolymphocyte	6-9 13
				Same or smaller	Negative	Lymphocyte	20-22 25-27

The numbers refer to the illustrations of the cells in Osgood E E and Ashworth Clarence M Atlas of Hematology Pp 255 J W Stacey Inc San Francisco 1937 from which this table is reproduced by permission of the copyright owners

† May or may not contain hemoglobin. Other nucleated cells never contain hemoglobin

‡ Sometimes two or more nuclei in one cell

(2) The red cells should be stained orange or buff colored. If normal red cells stain bluish or greenish, the fault is usually due to the use of water instead of buffer phosphate, to contamination of the buffer phosphate with alkali or it may be due to overstaining and the time should be decreased. Overstaining or precipitate on the slide may be corrected by treating the stained smear with 90 per cent ethyl alcohol saturated with Wright's stain (0.2 per cent)¹ and washing with water.

¹ Leemster, R J. A Study of the Variable Factors in the Use of the Wright's Stain. Ann Int Med 2 82-294 (Sept.) 1929. The method described in this article is satisfactory for routine use but is especially valuable for restaining old and faded or poorly stained preparations.

freeing the segments into the blood stream. In one half to one hour these will have entered other red cells and be starting the cycle over again. These segments are called merozoites and correspond to the hyaline form. The chill begins a few hours after the first segments appear. The pigment set free in the blood stream is taken up by the monocytes, hence the presence of an increased number of monocytes, many of them containing brownish pigment granules, is very suggestive of malaria. A diagnosis of malaria, however, should not be made without finding the parasite.

(6) Sexual forms. These are called gametes, and are often extra cellular. They are much less numerous than the intracellular asexual forms known as schizonts which have just been described. Detailed descriptions of these will be found in texts on parasitology. They are round or oval and larger than the asexual forms.

(c) *Double Tertian Malaria*—In this form, two stages of the *Plasmodium vivax* are present at the same time.

(d) *Quartan Malarial Parasite, or the Plasmodium Malariae*—This has a life cycle of 72 hours. It is the rarest form of malaria.

(1) The hyaline and ring forms are similar to those of the tertian parasite.

(2) The young pigmented forms differ from the tertian in that the pigment is in coarse clumps at the periphery of the organism and the red cell is not enlarged. They are seen 24 hours after the chill and have a tendency to assume a band form.

(3) The adult form appears on the third day. It is smaller than the tertian parasite and almost completely fills the red cell which is normal or shrunken in size. The cytoplasm of the red cell is darker than normal instead of paler as in the tertian type. The pigment remains at the periphery of the organism. Schueffner's granules are not seen in quartan infections.

(4) The segmented form. There are 6 to 12 segments (usually 8) which rupture the red cell just before the chill. They are often grouped in a regular rosette or daisy pattern. The pigment now clumps in the center or in star like radiations.

(5) The sexual forms differ from those of the tertian type only in the more peripheral distribution of pigment and their smaller size.

(e) *The Tropical or Estivo autumnal Malarial Parasite Called the Plasmodium Falciparum*—This is less common than the tertian and more common than the quartan type. It has a variable life cycle resulting in daily chills or chills at irregular intervals.

(1) Only the early stages of the schizonts appear in the peripheral blood stream. They are ring or signet ring in form and similar to the tertian ring forms but differ from the other forms in that there is greater tendency for two or more parasites to occur in a single red cell.

(2) Older parasites may be found in blood taken from the spleen, or sternal marrow but segmented forms rarely appear in the peripheral blood.

(3) Sexual forms. These may be found in the peripheral blood from about the seventh day after infection, and may persist even after all fever has subsided. They are more numerous than the sexual forms of other malarial parasites and have a characteristic ovoid or crescentic shape which is diagnostic of this type of malaria.

Note Stained blood smears on slides without cover slips may be preserved for several years. Immersion oil is removed by pouring benzol over the surface and wiping it off gently with the side of the hand or, better by placing a strip of lens paper over the slide, pouring the benzol on it, and pulling it off parallel with the plane of the slide. The slide method is much simpler and more satisfactory than the cover slip technic.

B Special Hematology—1. **Red Cell Volume Determination**. This should be done in all cases of anemia.

(a) *Technic*—Into a volume index tube¹ (Fig. 24) place about 4 cc of well mixed oxalated blood² and centrifugate at over 3000 revolutions per minute for about 2 minutes. Stop the centrifuge and read the total volume of blood, estimating hundredths of a cubic centimeter. This step in the procedure allows an accurate determination of the volume of blood taken, before evaporation has taken place. Then continue



FIG. 24

¹ These may be prepared by sealing off with heat the tip of a 10 cc Mohr pipette graduated to the tip, cutting it off above the 6 cc mark so that the capacity is approximately 4 cc and recalibrating it. More perfect tubes containing 4.5 cc and calibrated in 0.1 cc may now be secured from the Arthur H. Thomas Co., Philadelphia. The tubes are supported in a brass centrifuge tube holder by two rubber stoppers, one in the bottom of the holder indented to receive the tip of the tube and the other bored to fit the upper part of the centrifuge tube and flanged or glued in to prevent its being forced down into the holder during centrifugation or the upper part of the tube may be wrapped with rubber bands.

Wintrobe has also developed a satisfactory volume index tube which may be used as a sedimentation tube as well.

² It is important to have exactly 2 mg of oxalate per 1 cc of blood in the blood for a volume index determination because of the variation in red cell volume with changes in tonicity of the plasma. There is a decrease of about 3.5 per cent in the volume of the red cells due to this amount of oxalate but since the normal standards given in this book were obtained with the same technic no corrections are necessary. It is necessary to make corrections when comparing results by this method with results on bloods with which an isotonic anticoagulant solution was used.

the centrifugation over successive ten minute periods, noting each time the volume of the packed red cells, until two successive readings show no further decrease in the volume. This centrifugation to constant volume, not for a constant time period, is absolutely essential if cell volume, not cell sedimentation rate, is to be determined. From the figures obtained, calculate the volume of packed red cells per 100 cc of blood as follows: cubic centimeters of packed red cells divided by cubic centimeters of blood multiplied by 100.

2 **Calculation of the Color, Volume, and Saturation Indexes** — Consult Chapter VI for discussion and interpretation of these indexes and for the method of calculation of corpuscular hemoglobin, volume and hemoglobin concentration.

(a) *Color Index* — An accurate red cell count and hemoglobin estimation on the patient's blood and normal standards for the patient's sex and age group are necessary for the calculation. Five million is taken as 100 per cent red cells for both sexes and the average normal hemoglobin coefficient is taken as 100 per cent hemoglobin. Since the hemoglobin coefficient is in grams per 100 cc it is necessary to convert the patient's per cent figure to grams for comparison. Then, by the definition of the term "color index," it is necessary to convert both red cell count and hemoglobin into per cent of the normal standard.

The patient's red cell count divided by five million and multiplied by 100, or expressed as millions and multiplied by 20, gives the patient's percentage of red cells for use in calculation of indexes. The patient's hemoglobin percentage divided by 100 and multiplied by 13.8 if the Haskins Sahli or Osgood-Haskins methods were used gives the grams of hemoglobin per 100 cc of blood. If another hemoglobin method is used, substitute for 13.8 the number of grams of hemoglobin per 100 cc of blood that is 100 per cent in the method used. This figure divided by the normal hemoglobin coefficient of 14.7 for men, 14.3 for women, or 12.0 for children and multiplied by 100 gives the patient's per cent hemoglobin for use in the calculation of the color index.

$$\frac{\text{Per cent hemoglobin}}{\text{Per cent red cells}} = \text{Color index}$$

Tables 43 and 44 and chart II on pages 489 to 494 greatly simplify these calculations.¹ An example of their use is given

¹ Osgood E. E. Tables for Calculation of Color Index, Volume Index and Saturation Index Based on Recently Determined Standards. *J. Lab. and Clin. Med.*, 12: 899-906 (June) 1927. The table, chart and excerpts from this article are here reproduced by permission of the C. V. Mosby Company, St. Louis, Mo.

TABLE 43—SIMPLIFIED CALCULATION OF INDEXES

1	2	3 Men	4	5 Women	6	7
Per cent	Red cell count millions per cubic millimeter	Hemoglobin grams per 100 cc	Volume of packed cells cc per 100 cc	Hemoglobin grams per 100 cc	Volume of packed cells cc per 100 cc	Hemoglobin grams per 100 cc
10	0.50	1.47	4.10	1.43	4.30	1.38
11	0.55	1.62	4.51	1.57	4.73	1.52
12	0.60	1.76	4.92	1.71	5.16	1.66
13	0.65	1.91	5.33	1.86	5.59	1.79
14	0.70	2.06	5.74	2.00	6.02	1.93
15	0.75	2.21	6.15	2.15	6.45	2.07
16	0.80	2.35	6.56	2.29	6.88	2.21
17	0.85	2.50	6.97	2.43	7.31	2.35
18	0.90	2.65	7.38	2.57	7.74	2.48
19	0.95	2.79	7.79	2.72	8.17	2.62
20	1.00	2.94	8.20	2.86	8.60	2.76
21	1.05	3.09	8.61	3.00	9.03	2.90
22	1.10	3.23	9.02	3.15	9.46	3.04
23	1.15	3.38	9.43	3.29	9.89	3.17
24	1.20	3.53	9.84	3.43	10.32	3.31
25	1.25	3.67	10.25	3.58	10.75	3.45
26	1.30	3.82	10.66	3.72	11.18	3.59
27	1.35	3.97	11.07	3.86	11.61	3.73
28	1.40	4.12	11.48	4.00	12.04	3.86
29	1.45	4.26	11.89	4.15	12.47	4.00
30	1.50	4.41	12.30	4.29	12.90	4.14
31	1.55	4.56	12.71	4.43	13.33	4.28
32	1.60	4.70	13.12	4.58	13.76	4.42
33	1.65	4.85	13.53	4.72	14.19	4.55
34	1.70	5.00	13.94	4.86	14.62	4.69
35	1.75	5.15	14.35	5.00	15.05	4.83
36	1.80	5.29	14.76	5.15	15.48	4.97
37	1.85	5.44	15.17	5.29	15.91	5.11
38	1.90	5.59	15.58	5.43	16.34	5.24
39	1.95	5.73	15.99	5.58	16.77	5.38
40	2.00	5.88	16.40	5.72	17.20	5.52
41	2.05	6.03	16.81	5.86	17.63	5.66
42	2.10	6.17	17.22	6.01	18.06	5.80
43	2.15	6.32	17.63	6.15	18.49	5.93
44	2.20	6.47	18.04	6.29	18.92	6.07
45	2.25	6.62	18.45	6.44	19.35	6.21
46	2.30	6.76	18.86	6.58	19.78	6.35
47	2.35	6.91	19.27	6.72	20.21	6.49
48	2.40	7.06	19.68	6.86	20.64	6.62
49	2.45	7.20	20.09	7.01	21.07	6.76
50	2.50	7.35	20.50	7.15	21.50	6.90
51	2.55	7.50	20.91	7.29	21.93	7.04
52	2.60	7.64	21.32	7.44	22.36	7.18
53	2.65	7.79	21.73	7.58	22.79	7.31
54	2.70	7.94	22.14	7.72	23.22	7.45
55	2.75	8.08	22.55	7.87	23.65	7.59
56	2.80	8.23	22.96	8.01	24.08	7.73
57	2.85	8.38	23.37	8.15	24.51	7.87
58	2.90	8.53	23.78	8.29	24.94	8.00
59	2.95	8.67	24.19	8.44	25.37	8.14
60	3.00	8.82	24.60	8.58	25.80	8.28
61	3.05	8.97	25.01	8.72	26.23	8.42
62	3.10	9.11	25.42	8.87	26.66	8.56
63	3.15	9.26	25.83	9.01	27.09	8.69
64	3.20	9.41	26.24	9.15	27.52	8.83
65	3.25	9.55	26.65	9.30	27.95	8.97
66	3.30	9.70	27.06	9.44	28.38	9.11
67	3.35	9.85	27.47	9.58	28.81	9.25
68	3.40	10.00	27.88	9.72	29.24	9.38
69	3.45	10.14	28.29	9.87	29.67	9.52
70	3.50	10.29	28.70	10.01	30.10	9.66
71	3.55	10.44	29.11	10.15	30.53	9.80
72	3.60	10.58	29.52	10.30	30.96	9.94
73	3.65	10.73	29.93	10.44	31.39	10.07
74	3.70	10.88	30.34	10.58	31.82	10.21
75	3.75	11.03	30.75	10.73	32.25	10.35
76	3.80	11.17	31.16	10.87	32.68	10.49
77	3.85	11.32	31.57	11.01†	33.11	10.63
78	3.90	11.47	31.98	11.15	33.54	10.76
79	3.95	11.61	32.39	11.30	33.97	10.90
80	4.00	11.76	32.80	11.44	34.40	11.04
81	4.05†	11.91	33.21	11.58	34.83	11.18
82	4.10	12.05	33.62	11.73	35.26†	11.32
83	4.15	12.20	34.03	11.87	35.69	11.45
84	4.20	12.35	34.44	12.01	36.12	11.59

TABLE 43—SIMPLIFIED CALCULATION OF INDEXES—(Continued)

1	2	3	Men 4	5	Women 6	7
Per cent	Red cell count millions per cubic milli meter	Hemoglobin grams per 100 cc	Volume of packed cells cc. per 100 cc	Hemoglobin grams per 100 cc	Volume of packed cells cc per 100 cc	Hemoglobin grams per 100 cc
85	4.25	12.49	34.85	12.16	36.55	11.73
86	4.30	12.64	35.20	12.30	36.98	11.87
87	4.35	12.79	35.67	12.44	37.41	12.01
88	4.40*	12.94	36.08*	12.58	37.84	12.14
89	4.45	13.08	36.49	12.73	38.27	12.28
90	4.50	13.23	36.90	12.87	38.70	12.42
91	4.55	13.38	37.31	13.01	39.13	12.56
92	4.60	13.52*	37.72	13.16	39.56	12.70
93	4.65	13.67	38.13	13.30	39.99	12.83
94	4.70	13.82	38.54	13.44	40.42	12.9
95	4.75	13.96	38.95	13.59	40.85	13.11
96	4.80††	14.11	39.36	13.73††	41.28††	13.25
97	4.85	14.26	39.77	13.87	41.71	13.39
98	4.90	14.41	40.18	14.01	42.14	13.52
99	4.95	14.55	40.59	14.16	42.57	13.66
100	5.00	14.70	41.00	14.30	43.00	13.80
101	5.05	14.85	41.41	14.44	43.43	13.94
102	5.10	14.99	41.82	14.59	43.86	14.08
103	5.15	15.14	42.23	14.73	44.29	14.21
104	5.20	15.29	42.64	14.87	44.72	14.35
105	5.25	15.44	43.05	15.01	45.15	14.49
106	5.30	15.58	43.46	15.16	45.58	14.63
107	5.35	15.73	43.87	15.30	46.01†	14.77
108	5.40**	15.88**	44.28	15.44	46.44	14.90
109	5.45	16.03	44.69**	15.59	46.87	15.04
110	5.50	16.17	45.10	15.73	47.30	15.18
111	5.55†	16.32	45.51	15.87	47.73	15.32
112	5.60	16.46	45.92	16.02	48.16	15.46
113	5.65	16.61	46.33	16.16	48.59	15.59
114	5.70	16.76	46.74	16.30	49.02	15.73
115	5.75	16.90	47.15	16.44†	49.45	15.87
116	5.80	17.05	47.56	16.59	49.88	16.01
117	5.85	17.20	47.97	16.73	50.31	16.15
118	5.90	17.35	48.38	16.87	50.74	16.28
119	5.95	17.49	48.79	17.02	51.17	16.42
120	6.00	17.64	49.20	17.16	51.60	16.56
121	6.05	17.79	49.61	17.30	52.03	16.70
122	6.10	17.93	50.02	17.45	52.46	16.84
123	6.15	18.08	50.43	17.59	52.89	16.97
124	6.20	18.23	50.84	17.73	53.32	17.11
125	6.25	18.37	51.25	17.88	53.75	17.25
126	6.30	18.52	51.66	18.02	54.18	17.39
127	6.35	18.67	52.07*	18.16	54.61	17.53
128	6.40*	18.82	52.48	18.30	55.04	17.66
129	6.45	18.96*	52.89	18.45	55.47	17.80
130	6.50	19.11	53.30	18.59	55.90	17.94
131	6.55	19.26	53.71	18.73	56.33	18.08
132	6.60	19.40	54.12	18.88	56.76	18.22
133	6.65	19.55	54.53	19.02	57.19	18.35
134	6.70	19.70	54.94	19.16	57.62	18.49
135	6.75	19.84	55.35	19.30	58.05	18.63
136	6.80	19.99	55.76	19.45	58.48	18.77
137	6.85	20.14	56.17	19.59	58.91	18.91
138	6.90	20.29	56.58	19.73	59.34	19.04
139	6.95	20.43	56.99	19.88	59.77	19.18
140	7.00	20.58	57.40	20.02	60.20	19.32
141	7.05	20.73	57.81	20.16	60.63	19.46
142	7.10	20.87	58.22	20.31	61.06	19.60
143	7.15	21.02	58.63	20.45	61.49	19.73
144	7.20	21.17	59.04	20.59	61.92	19.87
145	7.25	21.31	59.45	20.73	62.35	20.01
146	7.30	21.46	59.86	20.88	62.78	20.15
147	7.35	21.61	60.27	21.02	63.21	20.29
148	7.40	21.76	60.68	21.16	63.64	20.42
149	7.45	21.90	61.09	21.31	64.07	20.56
150	7.50	22.05	61.50	21.45	64.50	20.70

The average figures for red cell count, hemoglobin estimation, and volume of packed red cells in the series of healthy men that we recently reported are indicated in the table by ** and the lowest and highest values found are indicated by †. Similarly the averages and extremes of variation found in the study of the bloods of 100 healthy young women are indicated by †† and † respectively.

TABLE 44—SIMPLIFIED CALCULATION OF INDEXES IN CHILDREN

1	8 Children 9	1	8 Children 9		
Per cent	Hemoglobin grams per 100 cc	Volume of packed cells per 100 cc	Per cent	Hemoglobin grams per 100 cc	Volume of packed cells per 100 cc
10	1 20	3 60	66	7 92	23 76
11	1 32	3 96	67	8 04	24 12
12	1 44	4 32	68	8 16	24 48
13	1 56	4 68	69	8 28	24 84
14	1 68	5 04	70	8 40	25 20
15	1 80	5 40	71	8 52	25 56
16	1 92	5 76	72	8 64	25 92
17	2 04	6 12	73	8 76	26 28
18	2 16	6 48	74	8 88	26 64
19	2 28	6 84	75	9 00	27 00
20	2 40	7 20	76	9 12	27 36
21	2 52	7 56	77	9 24	27 72
22	2 64	7 92	78	9 36	28 08
23	2 76	8 28	79	9 48	28 44
24	2 88	8 64	80	9 60	28 80
25	3 00	9 00	81	9 72	29 16
26	3 12	9 36	82	9 84	29 52
27	3 24	9 72	83	9 96	29 88
28	3 36	10 08	84	10 08	30 24
29	3 48	10 44	85	10 20	30 60
30	3 60	10 80	86	10 32	30 96
31	3 72	11 16	87	10 44	31 32
32	3 84	11 52	88	10 56	31 68
33	3 96	11 88	89	10 68	32 04
34	4 08	12 24	90	10 80	32 40
35	4 20	12 60	91	10 92	32 76
36	4 32	12 96	92	11 04	33 12
37	4 44	13 32	93	11 16	33 48
38	4 56	13 68	94	11 28	33 84
39	4 68	14 04	95	11 40	34 20
40	4 80	14 40	96	11 52	34 56
41	4 92	14 76	97	11 64	34 92
42	5 04	15 12	98	11 76	35 28
43	5 16	15 48	99	11 88	35 64
44	5 28	15 84	100	12 00	36 00
45	5 40	16 20	101	12 12	36 36
46	5 52	16 56	102	12 24	36 72
47	5 64	16 92	103	12 36	37 08
48	5 76	17 28	104	12 48	37 44
49	5 88	17 64	105	12 60	37 80
50	6 00	18 00	106	12 72	38 16
51	6 12	18 36	107	12 84	38 52
52	6 24	18 72	108	12 96	38 88
53	6 36	19 08	109	13 08	39 24
54	6 48	19 44	110	13 20	39 60
55	6 60	19 80	111	13 32	39 96
56	6 72	20 16	112	13 44	40 32
57	6 84	20 52	113	13 56	40 68
58	6 96	20 88	114	13 68	41 04
59	7 08	21 24	115	13 80	41 40
60	7 20	21 60	116	13 92	41 76
61	7 32	21 96	117	14 04	42 12
62	7 44	22 32	118	14 16	42 48
63	7 56	22 68	119	14 28	42 84
64	7 68	23 04	120	14 40	43 20
65	7 80	23 40	121	14 52	43 56

TABLE 44—SIMPLIFIED CALCULATION OF INDEXES IN CHILDREN—(Continued)

Children			Children		
1	8	9	1	8	9
Per cent	Hemoglobin grams per 100 cc	Volume of packed cells per 100 cc	Per cent	Hemoglobin grams per 100 cc	Volume of packed cells per 100 cc
122	14 64	43 92	136	16 32	48 96
123	14 76	44 28	137	16 44	49 32
124	14 88	44 64	138	16 56	49 68
125	15 00	45 00	139	16 68	50 04
126	15 12	45 36	140	16 80	50 40
127	15 24	45 72	141	16 92	50 76
128	15 36	46 08	142	17 04	51 12
129	15 48	46 44	143	17 16	51 48
130	15 60	46 80	144	17 28	51 84
131	15 72	47 16	145	17 40	52 20
132	15 84	47 52	146	17 52	52 56
133	15 96	47 88	147	17 64	52 92
134	16 08	48 24	148	17 76	53 28
135	16 20	48 60	149	17 88	53 64
			150	18 00	54 00

(b) *Volume Index*—An accurate red cell count, red cell volume determination, and standards for comparison are necessary for the calculation. The normal volume coefficient for the patient's sex and age group is taken as 100 per cent volume. The patient's red cell per cent is determined as described under color index. The volume of packed red cells per 100 cc of the patient's blood is determined. This figure divided by the normal volume coefficient of 41 for men, 43 for women, or 36 for children and multiplied by 100 gives the patient's per cent cell volume for use in the calculation of the volume index.

$$\frac{\text{Per cent cell volume}}{\text{Per cent red cells}} = \text{Volume index}$$

See Tables 43 and 44 and chart II for simplified calculation.

(c) *Saturation Index*—An accurate hemoglobin estimation and red cell volume determination are necessary for this calculation. Determine the per cent hemoglobin and cell volume in terms of the normal standard as described above.

$$\frac{\text{Per cent hemoglobin}}{\text{Per cent cell volume}} = \text{Saturation index}$$

This may also be calculated by dividing the color index by the volume index. See Tables 43 and 44 and chart II for simplified calculation.

(d) *Simplified Method for Index Calculation*¹—(1) Explanation of Table 43 To find the grams of hemoglobin per 100 cc, for any method using a content of 13.8 grams per 100 cc as 100 per cent look in column 7 opposite the per cent figure in column 1 To find the per cent red cells for index calculations read the figure in column 1 opposite the patient's red cell count figure (column 2) To find the per cent hemoglobin for index calculations read the figure in column 1 opposite the patient's grams of hemoglobin in column 3, 5 or 8 To find the per cent cell volume for index calculations read the figures in column 1 opposite the patient's cell volume in column 4, 6 or 9

(2) Explanation of the chart The chart is so designed that the vertical line corresponding to the intersection of any two printed lines of the logarithmic paper gives the quotient of the value indicated by the figure in the right hand column (X) divided by the value indicated by the figure in the left hand column (Y) Hence, it can be used for the determination of all of the indexes if one simply remembers always to look up the numerator of the fraction expressing the index in the right hand column (X) and the denominator in the left hand column (Y) A slide rule is even more satisfactory for index calculations

Readings from the chart are made easier by placing on it a sheet of transparent celluloid such as washed X ray film having 3 lines drawn on it carefully (Fig 25) two forming a right angle the third exactly bisecting the right angle and extending some distance beyond the apex of the angle

Line A is placed at the proper figure in the right hand column of the chart line B at the proper figure in the left hand column, and the final reading is made from the top of the chart at the point where line C lies

(3) Example of the calculation The study of the blood of Mrs F gave the following results

Red blood cells 1.62 million

Hemoglobin 52.0 per cent (Haskins Sahli method)

Volume of packed red cells 18.89 cc per 100 cc of blood (by the author's technic)

Reference to column 2 of the table shows that this red cell count corresponds to 32 per cent as read in column 1, of 5.0 million red cells

Reference to columns 1 and 7 shows that an estimation of 52.0 per cent *by this method* is equivalent to 7.18 gm of hemoglobin per 100 cc of blood

Then looking up 7.18 in column 5 since the patient is a woman, one finds in column 1 that it is 50 per cent of the normal hemoglobin coefficient for women

In the same manner looking up 18.89 cc in column 6 one finds in column 1 that it is 44 per cent of the normal volume coefficient for women

Now determine the indexes by use of Chart II The color index is $50/32$ Therefore look up line 50 in column X and line 32 in column Y, and find that they

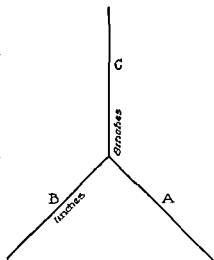
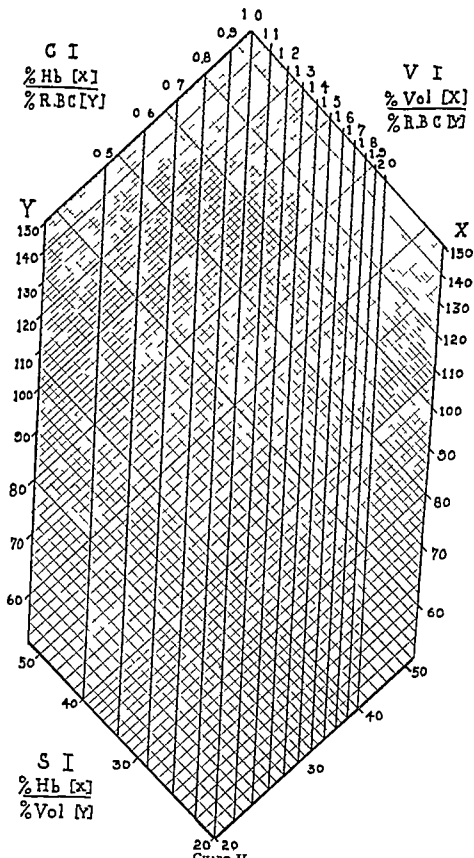


FIG 25

¹ See footnote 1 page 488



intersect about midway between vertical lines 15 and 16, corresponding to a color index of 1.55

Similarly the volume index is $44/32$. These lines intersect at a vertical line corresponding to a volume index of 1.36

The saturation index is $50/44$ which from the chart is found to be 1.14

The laboratory report on this case would then read

Red blood cells 1.62 million

Hemoglobin 7.2 grams (52.0%)

Color index 1.55

Volume index 1.36

Saturation index 1.14

3 **Peroxidase Stain**—The Washburn¹ modification of the Goodpasture stain with minor improvements is recommended

(a) *Technic*—Thin air dried smears should be made within 3 to 4 hours after drawing the blood and should be stained within 12 hours unless carefully protected from light and air. Place on the slide 10 drops of solution I and allow it to stand 1 to $1\frac{1}{2}$ minutes; add directly to this 5 drops of solution II and allow it to stand 3 to 4 minutes, wash thoroughly in tap water for 1 to $1\frac{1}{2}$ minutes and air dry. Then add 8 drops of *Wright's stain*, allow to stand 3 to 4 minutes; add 12 drops of *buffer phosphate solution* and allow to stand 20 to 45 minutes. Leukemic bloods and sternal marrow require a longer time. Wash the slide as directed for the Wright's stain and air dry.

(b) *Description of the Stain*—The nuclei of all cells stain as usual with Wright's stain and therefore aid in their identification. The neutrophils are filled with large black granules. The eosinophils contain very large dark granules which are refractile and have a paler brownish center with darker periphery. The basophils have large black granules which tend to concentrate at the periphery of the cell. Lymphocytes show no granules and so appear as in ordinary smears. Monocytes have dark, rod shaped granules which are not numerous and which tend to clump together. Blast cells show no granules. Red cells and platelets stain as usual with Wright's stain. The progranulocytes (promyelocytes) show few to many dark greenish black granules which differentiate them from polymorphocytes which show no granules and stain as in the Wright's stain. One should examine all cells with round nuclei which are larger than the lobocytes (polymorphonuclears) in order to determine whether the characteristic pro cell present is a progranulocyte (promyelocyte) or polymorphocyte.

(c) *Reagents*—(1) **Solution I** Benzidine (0.3 gm) is dissolved in 99 cc of ethyl alcohol then 1 cc of saturated (36 per cent) sodium nitroprusside solution is added. This stain keeps for 8 to 10 months.

(2) **Solution II** Five to six drops of hydrogen peroxide in 25 cc of water. This solution should not be over 48 hours old.

4 **Determination of Red Cell Diameter**—Many methods² have been devised for this purpose. Two hundred to 1000 cells may be measured with an eye piece micrometer with a filar micrometer by projection and direct measurement, by

¹ Washburn A. H. A Combined Peroxidase and Wright's stain for Routine Blood Smears. *J. Lab. and Clin. Med.* 14: 246-250 (Dec.) 1928.

² Christophers S. R. and Craighead A. C. The Diffraction (Halographic) Method of Determining the Average Diameter of Red Blood Corpuscles. *Ind. J. Med. Research* 19: 933-96 (Jan.) 1937. See also the references on page 190.

tracing the projected image, by measurement of photomicrographs, or the average cell diameter may be determined by the use of the diffraction principle. Any of these methods may be used with dry or moist preparations and the dry smears may be examined unstained or fixed and stained. The methods discussed below are the ones the author recommends as most practical clinically. The others will be found discussed in the references cited.

(a) *Eye piece Micrometer*—Select an area on a thin smear, stained with Wright's stain, where none of the red cells touch each other and there is no crenation. Measure 200 to 1000 consecutive red cells with the eye piece micrometer and record the results to the nearest 0.1 micron (for directions, see page 331). Plot a curve showing the percentage of red cells found of each diameter. If the mechanical stage is moved in one direction and all cells are measured in the diameter on which they happen to fall on the micrometer scale regardless of shape, the laws of chance will automatically average the cell diameters so that it is unnecessary to measure each individual cell in more than one diameter. This method is very laborious and only in hemolytic icterus and in rare cases of pernicious anemia with many microcytes does it add any to the information derived from the simple and more accurate volume index determination.

(b) *The Diffraction Method*¹—Determine the average cell diameter on a thin unstained and unfixed blood smear by use of an eriometer or a halometer. The directions for making these instruments are given in the articles cited.² These instruments are extremely simple to use. The estimation ordinarily does not require over a minute but gives only the average cell diameter and is difficult or impossible to read in anemias associated with marked anisocytosis. Directions accompany the instruments. In all of them, concentric circles of rainbow colors are produced by the diffraction of light, each of which is inversely proportional in diameter to the diameter of the red cells.

5 Reticulocyte Staining and Enumeration—The method in most common use is to spread a saturated alcoholic solution of brilliant cresyl blue on a slide, allow this to dry, make a smear of blood over it, and counterstain with Wright's stain. The reticulocytes appear well stained but many are not stained at all so it is not recommended.

(a) *Osgood Wilhelm Method*³—This method is recommended. Mix in a small test-tube, equal parts (5 drops) of oxalated venous, or capillary⁴ blood and 10 per cent brilliant cresyl blue in 0.85 per cent sodium chloride solution. Let stand at least one minute, mix, and make thin smears, drying in the air as usual. These smears may be counted.

¹ Emmons W. F. The Clinical Eriometer. *Quart J Med* 21: 83-90 (Oct) 1927.

Piper A. An Improved Diffraction Method for Diagnosing and Following the Course of Pernicious and Other Anaemias. *Brit Med J* 1: 635-638 (April 6) 1929.

Pryce D. M. A Simplification of the Halo Method of Measuring the Diameter of Red Blood Corpuscles. *Lancet* 2: 275-276 (Aug 10) 1929.

Eve F. C. The Early Diagnosis of Pernicious Anaemia by the Halometer. *Brit Med J* 2: 48-49 (July 13) 1929.

² A satisfactory eriometer designed and manufactured by A. H. Osgood can be obtained from the Shaw Surgical Company, Portland, Ore.

³ Osgood E. E., and Wilhelm Mable M. Reticulocytes. *J Lab and Clin Med* 19: 1129-1135 (July) 1934.

⁴ The blood and stain may be mixed in a white cell diluting pipette.

at any time. They may be counterstained with Wright's stain by the usual technic if desired. The brilliant cresyl blue solution keeps well but should be filtered if a precipitate appears on the smear.

Select an area on the slide which contains 50 to 75 red cells per oil immersion field and count all the red cells and all the reticulocytes in as many adjacent fields as is necessary to give a total of 500 red cells, if the count is over 5 per cent. If the count is less than 5 per cent, 1000 cells should be counted.

This method has the following advantages over those in common use. It was determined by actual experiment that this technic gives the optimum conditions for reticulocyte staining, it shows about three times as many reticulocytes as other methods in common use, and it is not necessary to counterstain with Wright's stain.

(b) *Cover Slip Method*—Place a small drop of brilliant cresyl blue solution on a glass slide and then place a tiny drop of fresh blood on a cover slip over it. Rim it with vaseline and examine after 1 minute. The reticulocytes may be counted in this moist cover slip preparation. A permanent mount may be made by removing the cover slip (omit vaseline) with a lateral motion, drying the smear quickly and staining it with Wright's stain, using about one third the time after dilution as for a regular stain.

6 *Moist Cover Slip Preparations*—Clean slides and cover slips very thoroughly and keep them and 0.9 per cent saline in an incubator so that they will always be ready at the correct temperature. Holding the cover slip by its corners rim a band about 1 mm. wide around the edge with a thin layer of vaseline or immersion oil using a tooth pick to spread it. Place a tiny drop of freshly drawn blood in the center of the cover slip and press it on the slide firmly enough to make an air tight seal all the way around and to show under the microscope a red cell layer only one cell thick. Sometimes it is desirable to use a small drop of saline with the drop of blood. One must learn by practice the correct amount of blood to use to get a satisfactory preparation. This may be examined immediately for ameboid activity of the white blood corpuscles for malaria parasites or filaria larvae and after 24 hours standing for sickle cells.¹ The malaria parasites appear as hyaline bodies within the red cells with the pigment within them showing a rapid dancing movement. Filaria larvae are located by the motion of the red cells in their vicinity. They are about 10 micra wide and 300 micra long and keep up a rapid thrashing motion for hours. They appear in blood taken only at one time of the day or night. Sickle cells are most numerous after 24 hours and show multiple points with long filaments projecting from them (Fig. 6).

(a) *Supravital Preparations*—Make the mounts as described above for moist cover slip preparations on slides coated with dye as described below and examine after 15 minutes to 2 hours at room temperature or the slides may be left in an ice

¹ Diggs L. W. The Sickle Cell Phenomenon. I. The Rate of Sickling in Moist Preparations. J. Lab. and Clin. Med. 17: 913-920 (June) 1932.

box over night and examined the next day. A warm stage as originally described is not necessary.

With this technic, neutrophil granules stain pink, eosinophil granules stain yellowish, and basophil granules stain red. Mitochondria, which are not visible in Wright's stain, stain green. Nuclei have the same shapes as in the corresponding cell in the Wright's stain but are unstained if the cell is still living. The lobocytes (polymorphonuclears) and rhabdocytes (staff cells) are actively motile, showing rapid movement of the granules as well as ameboid motion. Vacuoles containing orange red dye are common in the monocytes and neutrophils. Mitochondria are rarely seen in cells of the granulocyte (myeloid) series more mature than the granuloblast (myeloblast) and progranulocyte (promyelocyte). None of the blast cells take up neutral red. Ameboid motion is rare in progranulocytes (promyelocytes) and granulocytes (myelocytes) but the neutral red granules may move. Lymphocytes contain a few scarce vacuoles containing neutral red and many mitochondria. They are more numerous in the lymphoblasts and prolymphocytes. The mitochondria of lymphocytes are larger than those of monocytes and tend to be located near the nucleus rather than at the periphery. In monocytes the neutral red vacuoles tend to be clumped in a rosette and the mitochondria are small and more numerous near the periphery of the cell. Monocytes and lymphocytes may show rounded projections from the cytoplasm with slow changes in shape but are much less actively motile than cells of the granulocyte (myeloid) series. If motility alone is to be studied, the Janus green may be omitted from the stain or the cells may be examined unstained under dark field illumination.

(1) Reagents.—Keep stock solutions of saturated neutral red and Janus green in absolute ethyl alcohol. From these, prepare at intervals dilute solutions. Add 20 to 30 drops of neutral red and a like amount of Janus green to 10 cc. of absolute ethyl alcohol. Mix. The Janus green may be omitted if desired.

(2) Cleaning glassware and applying stain.—In all manipulations, handle the slides and cover slips with forceps, not with the fingers. Wash thoroughly in soap and water, place in running tap water for several hours and rinse in distilled water. Leave in dichromate sulphuric acid cleaning mixture for a week or more and then wash with tap water and rinse with distilled water. Keep them in 70 per cent alcohol for two weeks or longer, dry with a clean old linen cloth and flame the slides but not the cover slips. Allow to cool and flood with the stain. Drain off the excess stain and stand the slides against the wall on a clean paper towel. As soon as they are dry mark the stained surface with a wax pencil and store in a dust proof slide box or wrap in paper. They keep indefinitely.

7 Fragility of the Red Blood Corpuscles.—Several methods are in use but the Giffen and Sanford¹ modification of Ribierre's method is recommended. Oxalated blood is preferable to capillary or untreated venous blood because it does not necessitate carrying the apparatus to the bedside and the change of tonicity due to the added oxalate is too small to be considered being only 0.0055 per cent calculated as sodium chloride solution. The test may be done on oxalated blood that has stood an hour or more with perfect results.

(a) *Giffen and Sanford Method*.—(1) Technic and reading of results. An accurate 0.5 per cent solution of sodium chloride is made by dissolving exactly 0.5 grams

¹ Giffen, H. Z. and Sanford, A. H. Fragility of Erythrocytes. *J. Lab. and Clin. Med.* 4: 465 (May) 1919.

of C P sodium chloride in distilled water and diluting to 100 cc. in a volumetric flask. A series of 12 narrow test tubes numbered down from 25 to 14 are placed in a test tube rack with a duplicate set behind for the control. Place the number of drops of 0.5 per cent sodium chloride solution indicated by the number (25, 24, etc.) in each tube and add a sufficient number of drops of distilled water to make the total 25. Multiples of 0.08 or 0.1 cc. may be measured with a pipette more quickly and accurately than tubes can be set up by the drop method. Fill the rear tubes in the same manner. Mix well. Add one drop of the patient's oxalated blood to each tube of the front row and one drop of oxalated blood from a normal person to each tube in the rear row. Shake the tubes to insure mixing and read the results after they have stood an hour or two at room temperature. The first tube showing a tinge of red in the supernatant liquid is the point of beginning hemolysis, the first point where no corpuscular residue is visible even after centrifugating is the point of complete hemolysis. The per cent is figured by multiplying the figure on the tube by 0.02. Thus the tube containing 14 drops of saline is 0.28 per cent.

It is sometimes necessary to use washed corpuscles. To secure these, prevent the blood from clotting by discharging 3 cc. into 5 cc. of 2 per cent sodium citrate solution (oxalated blood is also satisfactory) and by repeated centrifugating (3 times) and washing with 0.9 per cent sodium chloride solution, free the corpuscles of plasma. Use the final suspension in 3 cc. of saline in the same way as blood.

If concentrations above 0.50 per cent sodium chloride are desired, use an exact 1 per cent solution of sodium chloride and the same rule as above. The per cent is figured by multiplying the number on the tube by 0.04.

(b) *Fontaine Technic*—This is simpler but it detects only marked variations.

(1) *Technic*. Draw up blood to the 10 mark on a white cell pipette and empty it into 1 cc. of exact 0.9 per cent sodium chloride solution. Mix well and place 0.1 cc. of it in each of nine test tubes (4 by $\frac{1}{4}$ inch in size) containing distilled water and exact 0.9 per cent sodium chloride solution as follows:

Water	0.1	0.2	0.3	0.4	0.5	0.6	0.7	0.8	0.9 cc
0.9 per cent sodium chloride	0.8	0.7	0.6	0.5	0.4	0.3	0.2	0.1	0.0 cc
Per cent of NaCl (final)	0.81	0.72	0.63	0.54	0.45	0.36	0.27	0.18	0.09

Incubate the tubes for 1 hour at 37° C. and then read them. The reading is more accurate if they are allowed to settle in the ice box for 24 hours. Note the point of beginning hemolysis and of complete hemolysis.

8 Sternal Puncture¹ and Examination of the Sternal Marrow—The puncture itself should be done by a physician, but anyone capable of recognizing all of the cells seen in the blood in granulocytic (myelogenous) leukemia should be able to make the examination of the aspirated material.

(a) *Technic of the Puncture*—With the patient lying on his back and his chest elevated by a pillow beneath his shoulders, prepare the

¹Young R. H. and Osgood F. E. Sternal Marrow Aspirated During Life. *Cytology in Health and Disease*. Arch. Int. Med. 55: 186-203 (Feb.) 1935.

region of the sterno manubrial junction with iodine and alcohol. Using aseptic technic, locate the sterno manubrial junction as a distinct ridge opposite the sternal cartilages of the second ribs, and infiltrate the skin, subcutaneous tissues and periosteum of this region with procaine. Using a sternal puncture needle¹ or a 16 to 18 gauge spinal puncture needle, cut to 30 to 40 cm in length and rebevelled, enter the sterno manubrial junction in the mid line at an angle of about 60° to the surface of the chest. Then depress the needle to an angle of about 30° and rotate it until it enters the marrow cavity of the body of the sternum, taking care not to exceed a total depth of 15 cm. Remove the stylet and, using an *air tight* 10 cc Luer syringe, aspirate 1 or 2 cc of marrow. If no marrow appears after strong aspiration, replace the stylet, insert the needle a little deeper, and reaspirate. Remove the syringe from the needle and transfer the aspirated material, which looks like blood, into a 4 × ½ in test tube containing 2 or 4 mg of powdered potassium oxalate (p 461), shaking well to insure mixing. Replace the stylet, *withdraw the needle, and seal the puncture wound with collodion*. Use this oxalated marrow for any type of hematologic examination which can be done on oxalated blood.

(b) *Technic of Examination*—Make thin smears as directed for blood and stain with Wright's stain, using double the time after adding the buffer phosphate that is used for blood. Count 500 nucleated cells as in a differential cell count on blood, except that nucleated red cells are included. Calculate the percentage of each type. The same criteria are used for identification of the cells as are used for identification of these cells in the blood. The megalokaryocytes may be recognized by their large size, 40 to 60 micra, lobulated nuclei and blue cytoplasm containing purplish granules similar to those seen in platelets. A reticulocyte count and peroxidase stain are often desirable and may be done by the same technic as recommended for blood. A total nucleated cell count should be done by the technic given for a white cell count in the blood. A red cell count and hemoglobin estimation are possible, but add little of interest.

9 **Splenic Puncture**—The puncture itself should be done by a physician.

(a) *Technic of the Puncture*—With the patient lying on his back determine by percussion that there is no bowel between the enlarged spleen and the anterior abdominal wall. Prepare the area selected with alcohol and iodine and infiltrate with 1 per cent procaine down to the peritoneum. With the abdominal wall pressed firmly against

¹ Obtainable from Becton Dickinson & Co

the spleen so as to prevent its movement with respiration (instruct the patient to hold his breath or to breathe very shallowly), introduce an 18 to 20 gauge sterile needle attached to a sterile 10 cc syringe, just through the splenic capsule and aspirate strongly or until 1 cc of material is obtained. Then withdraw the needle and syringe while maintaining suction. Thin smears should be made at once, or if enough material is obtained, it may be mixed with oxalate as directed for marrow. The smears should be stained with Wright's stain as directed for blood, using double the time after adding the buffer phosphate. Examine the stained smears for immature leukocytes or erythrocytes and for malaria parasites and Leishman-Donovan bodies. Leishman Donovan bodies are blue staining objects about 2 micra in diameter containing a large and a small reddish chromatin mass within the endothelial cells. Look also for the large cells with a foamy cytoplasm characteristic of some forms of lipid histiocytosis.

10 Red Cell Sedimentation Rate¹—A large number of methods are now in use for this determination, all of which give satisfactory results if standards for comparison are first determined on a large number of normals and known pathologic cases. Many of these methods require carrying the apparatus to the bedside, which is a definite disadvantage, and often an extra venipuncture is done for the sole purpose of this determination. A few of the methods require an excessive amount of blood. To overcome these objections the Westergren² tubes and rack were adapted for use with oxalated venous blood. The results obtained on normal and pathologic bloods were almost identical with the two methods. Only two readings are taken, one at 15 and another at 45 minutes. The advantages of this method are the determination may be done at any time within 3 hours after venipuncture on the same blood that is used for blood chemistry and other hematologic methods, only two readings are taken and, therefore, very little attention is required, the apparatus is cheap and the pipettes are quickly and easily cleaned, a separate puncture is not necessary, errors from inaccurate dilution are eliminated, and a report is available within 45 minutes.

(a) *Modified Westergren Method*—(1) *Technic* Draw well mixed oxalated venous blood up to the zero mark on the pipette, wipe the excess blood off the tip, and insert in the rack (figure 26). Press the tip

¹Haskins H D, Trotman F E, Osgood E E and Mathieu A. A Rapid Method for Determination of the Sedimentation Rate of the Red Cells with Results in Health and Disease. J Lab & Clin Med. 16: 487-494 (Feb.) 1931.

²Westergren A. Technic of Red Cell Sedimentation Reaction. Am Rev Tuberc. 14: 94-101 (July) 1926.

of the pipette against the rubber before releasing the finger. The pipette should be exactly vertical. Note the upper level of the blood if it is not exactly at the zero mark and record the time, read the upper level of the red cells at 15 and 45 minutes after the start. In large laboratories an interval timer is a convenience.

(2) *Apparatus* Pipettes graduated from 0 to 200 mm at the tip may be obtained from the Arthur H. Thomas Co., Philadelphia (specifications No. 1710 C) or satisfactory substitutes can be made by selecting a number of 1 cc. pipettes

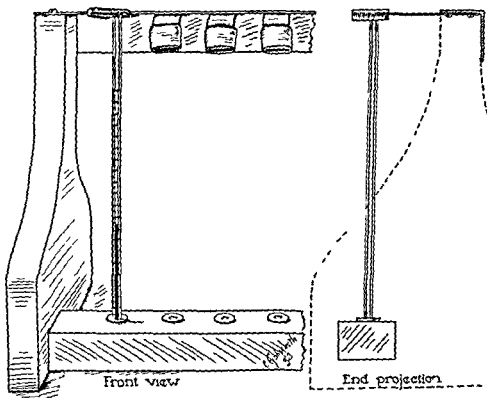


FIG. 26—Sedimentation rack and tube

which contain 1 cc. at a mark within a few mm. of 200 mm. from the tip and pasting a 150 mm. strip of mm. graph paper along the side of the pipette with the upper end exactly 200 mm. from the tip. Shellac over this to prevent soiling.

Have a rack¹ so made that, with the tip of the pipette resting on a rubber cork in the base, a rubber covered spring at the top will clamp down on the pipette sufficiently firmly to hold it in a vertical position and prevent leakage of blood. See Fig. 26.

(b) *The Lavenmeter Method*—This is one of the oldest methods and is probably the most widely used at the present time. The tubes are only 7 cm. long and 5 mm. in internal diameter and have to be watched closely until the cells reach the 18 mm. mark. The objections are the use of citrated blood (special solutions), the amount of attention required, and the difficulty of cleaning the tubes.

¹ An improved rack is obtainable from Braun Knecht Helman, San Francisco, or the Scientific Supplies Co., Seattle.

11 Bleeding Time (Duke) —If found prolonged, a platelet count is indicated

Technic —Clean the ear lobe with alcohol and allow it to dry. Make a puncture with a sharp lancet, collect the drops on blotting paper each half minute and note the time from the appearance of the first drop until the bleeding stops. The normal time for this method is 3 minutes or less.

12 Coagulation Time —The most accurate method and the one recommended is that of Lee and White.

(a) *Technic* —Take blood from the vein in a syringe that has been rinsed out with normal saline and run 1 cc immediately into each of 3 test tubes 8 mm in diameter which have also been rinsed out with normal saline. Every 15 seconds, tip the first tube slightly until the blood no longer flows and the tube can be inverted. When this occurs test the second in the same way and record the time when the second tube can be inverted as the coagulation time. The normal is 5 to 8 minutes and the average is $6\frac{1}{2}$ minutes. If the tube diameter is 9 mm the normal is 6 to 11 minutes. The third tube is used for the clot retraction test (see page 504).

The following two methods are given because they are frequently used but since tissue juice is mixed with the blood they may fail to show prolonged coagulation time even in hemophilia. In my opinion they are valueless.

Place a drop of blood on a clean glass slide and pass a needle or pin through it every half minute after the first 3 minutes. The slide may be supported on glass rods in a petri dish above a piece of blotting paper moistened with warm water to prevent drying. When a thread of fibrin is picked up by the needle it is said to have clotted. The normal is 7 minutes or less. If deviations are found by this method they should be checked by the Lee and White technic.

Another much used method is to draw out in the flame a supply of clean glass tubing to form capillary tubing 1 mm or less in diameter and in lengths of about 10 cm. Fill two or three of these tubes by capillarity from the third or later free flowing drop from a puncture made with a sharp knife. After the first 3 minutes, break off about 1 cm length of tubing each 30 seconds and record the clotting time as the interval from the time the drop appears on the skin surface until a fibrin thread attaches the broken ends until they have been separated a perceptible distance. The maximum normal coagulation time by this method is 7 minutes.

13 Clot Retraction Determination—*Technic*—The third tube secured in the coagulation test (above) should be placed in an incubator at 37°C and observed at 1 hour, 18 hours, and 24 hours. Normally retraction of the clot from the wall with separation of serum begins in 1 hour and is complete in 18 to 24 hours.

14 Platelet Count—*Technic*—Oxalated blood and freshly made or freshly filtered Toisson's diluting fluid must be used. The dilution

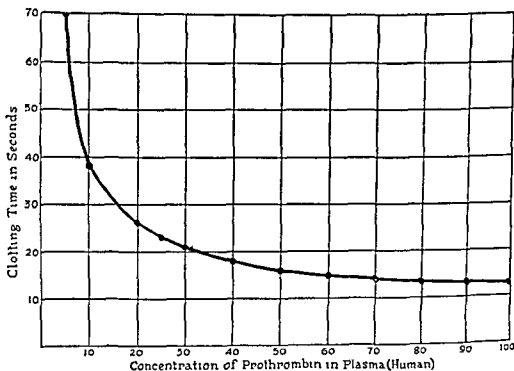


CHART III—The relation of prothrombin concentration to the clotting time of recalcified plasma containing an excess of thromboplastin.¹

of the blood and preparation of the mount is identical with that for the red cell count with these exceptions: the preparation in the counting chamber must stand 10 minutes before counting and the light must be reduced until the platelets appear as small highly refractile dots. Using the high power objective, count 4 of the sixteen large squares in each corner, making a total of 16 squares and an area of 1 sq. mm which is equivalent to a volume of 0.1 c. mm. The dilution is 1 to 200, so multiply the total count by 2000 to get the number in 1 c. mm.

All methods so far devised for platelet counting are very inaccurate. Another method is to count all the platelets seen in counting 1000 red cells in a thin smear of oxalated blood stained with Wright's stain, calculating the platelet count by proportion from the red cell count.

¹ See footnote 1 on page 505.

15 The Capillary Resistance Test of Rumpel-Leede —

Technic—Mark with a skin pencil any petechiae which may be present on the patient's arm or hand. Apply the cuff of a blood pressure apparatus in the usual way and maintain it at the diastolic pressure for 5 minutes. Record the number and site of any new petechiae which may develop while the cuff is in place or after its removal.

16 The Prothrombin Time (Quick¹)—(a) *Principle*—There is evidence to suggest that the clotting time of recalcified plasma containing an excess of thromboplastic substance of which cephalin is the important constituent bears a relation to the level of prothrombin present in the plasma.

(b) *Technic*—Withdraw 4.5 cc of blood by venipuncture, mix immediately with 0.5 cc of sodium oxalate solution and centrifugate.

Mix 0.1 cc of plasma with 0.1 cc of thromboplastic solution and quickly add 0.1 cc of calcium chloride solution. Record accurately the time required for the formation of a clot after the addition of the calcium chloride. Normal plasma will clot in from 12 to 13 seconds. With a decrease in prothrombin, the clotting time is delayed. By means of Chart III determine the prothrombin content of blood from

TABLE 45—NORMAL VALUES FOR HEMATOLOGIC METHODS¹

	Males	Females
Red blood cells—average	5.40 million	4.80 million
Range	4.40 to 6.4 million	4.0 to 5.6 million
Total hemoglobin—average (per 100 cc)	15.8 grams	13.7 grams
Range	13.5 to 19 grams	11.0 to 16.5 grams
Hemoglobin coefficient	14.7	14.3
Volume coefficient	41.0	43.0
White blood cells	4.5 to 11.5 thousand	4.5 to 11.5 thousand
Neutrophil lobocytes	33 to 75 per cent	33 to 75 per cent
Red cell diameter	6 to 9 micra	6 to 9 micra
Reticulocytes	0.5 to 3.0 per cent	0.5 to 3.0 per cent
Red cell fragility—beginning hemolysis	0.46–0.38% NaCl	0.46–0.38% NaCl
Complete hemolysis	0.36–0.30% NaCl	0.36–0.30% NaCl
Sedimentation rate—first 15 minutes	0.0 to 5.0 mm	0.0 to 5.0 mm
Total 15 minutes	1.0 to 30.0 mm	1.0 to 30.0 mm
Bleeding time (Duke's method)	3 minutes	3 minutes
Coagulation time (Lee and White)	5 to 8 minutes	5 to 8 minutes
Clot retraction (Lee and White)	Beginning in 1 hr	Beginning in 1 hr
	Complete in 18–24	Complete in 18–24
Platelet count	250,000 to 450,000	250,000 to 450,000

¹ See also Table 7 and figures 3, 4, and 7.

¹ Reproduced by permission of the author and the publishers from Quick, A. J. The Nature of the Bleeding in Jaundice. J. A. M. A. 110: 1658–1662 (May 14) 1938.

the clotting time As an example, if the plasma clots in 31 seconds, the prothrombin is 15 per cent of the normal

(c) *Reagents* —(1) Sodium oxalate dissolve 1.34 gm of anhydrous pure sodium oxalate in 100 cc of distilled water

(2) Calcium chloride dissolve 1.11 gm of anhydrous chemically pure calcium chloride in 400 cc of distilled water

(3) Thromboplastic solution mix 0.3 gm of dehydrated rabbit brain with 5 cc of physiologic solution of sodium chloride containing 0.1 cc of sodium oxalate Incubate at 45° C for 10 minutes, then centrifugate at a slow speed for 3 minutes to obtain a milky supernatant liquid free from coarse particles

The rabbit brain is dehydrated as follows after the blood vessels have been carefully removed by stripping off the pia, the brain is macerated in a mortar and extracted with acetone The solvent is poured off and a fresh amount of acetone added The process is repeated until a granular powder is obtained The product is dried at 37° C and placed in a stoppered container and preserved in a refrigerator It retains its full activity for a week It is advisable to check the activity of the thromboplastin against a normal plasma

SECTION X SIMPLE BACTERIOLOGIC AND SEROLOGIC TECHNIC

No attempt is made to give the morphology or cultural characteristics of even the most important organisms and only a very few of the commonly used methods will be reviewed here as this work is given in detail in other courses in the medical curriculum. The standard texts on these subjects should be referred to for details.

A Bacteriologic Methods—1 **Making and Fixing Smears**—The material to be examined should be carefully selected in such a way as to avoid as far as possible contamination with other material. In wounds cervical or urethral smears etc. wipe off the first exudate expressed and collect the material on a second applicator. In throat smears be certain that the applicator touches no area but that from which the smear is desired. In sputum select caseous or purulent masses with care. A sterile cotton applicator which should be rolled, not wiped over the exudate and then rolled, not wiped over the slide is most satisfactory, except for very fluid material. For the latter for sputum or pus, and for cultures a platinum loop sterilized by flaming before and after use is preferable. Add a loopful of water if necessary and spread the exudate in a thin layer on the slide. Make at least three smears from each source: one for Gram's stain, one for methylene blue stain and the third for a recheck or for a special stain. Allow them to dry in the air and fix by passing them film side up, slowly through a flame 10 to 12 times. Label with the nature of material such as pus discharge, or membrane and its source, such as cervix urethra right tonsil or sinus in left cheek etc. in addition to the tentative diagnosis or organisms sought, as gonococcus Vincent's organisms, actinomycosis, or diphtheria, and the usual data as to date name and case number.

Urine spinal, pleural or ascitic fluid should be collected with aseptic precautions into sterile centrifuge tubes and centrifugated slowly for one minute to throw down leukocytes and other cells. Stain smears of this sediment for cytology and for intracellular organisms. The supernatant fluid should then be decanted into a second sterile centrifuge tube and centrifugated at 3500 revolutions per minute or over for 30 minutes or longer. Dilution with two volumes of alcohol before centrifugation lowers the specific gravity and increases somewhat the percentage of positive results. Make and stain smears from this sediment for bacteria and particularly for the tubercle bacillus. Blood serum may be necessary to make it stick to the slide. This sediment may be used also for guinea pig inoculation. Only positive results are significant.

A still better method is to treat 10 cc. of the sediment obtained from a large volume of urine or puncture fluid with an equal volume of 30 per cent antiformin and proceed as outlined under sputum examination page 516.

2 **Staining Methods**—(a) *Loeffler's Methylene Blue*—Make heavy wax pencil marks to limit the stain to the desired area and cover with the stain. After 30 seconds for most organisms or 5 minutes or over for diphtheria or Vincent's organisms¹ wash in water and allow to dry in the air. This is a very satisfactory stain.

¹ Gentian violet or carbolfuchsin are better than methylene blue for staining Vincent's organisms.

for studying the morphology of organisms and should be used routinely in addition to the Gram's stain

Reagent Mix 30 cc of saturated alcoholic solution (0.75 grams per 100 cc of 95 per cent ethyl alcohol) of methylthionine chloride (methylene blue) and 100 cc of 1 to 10,000 aqueous potassium hydroxide solution. The solution improves on standing and keeps indefinitely.

(b) *Gram's Stain*—Cover the fixed smear with Stirling's gentian violet solution or Hucker's crystal violet solution. After 1 minute, wash in water. Cover with Gram's iodine solution for one minute. Rinse off the iodine with 95 per cent alcohol (1 part ether plus 3 parts acetone decolorizes faster but is more expensive) and decolorize in a staining jar of 95 per cent alcohol until no more purple color dissolves out and the smear becomes grayish. The nuclei of white cells should be unstained at this time if decolorization is complete. Wash in water, and counterstain 1 minute with 0.25 per cent safranin. Wash and allow to dry in the air.

Gram positive organisms are purplish blue, Gram negative are red.

Reagents Stirling's anilin gentian violet. Dissolve 2 cc of anilin in 10 cc of 95 per cent ethyl alcohol and shake, add 88 cc of distilled water and shake. Put 50 grams of gentian violet (crystal violet) in a mortar and add the anilin mixture slowly while grinding. Filter. This keeps indefinitely and much better than most other anilin gentian violet solutions.

Hucker's modification, recommended by a special committee of the American Society of Bacteriologists. Mix 20 cc of 20 per cent crystal violet (85 per cent dye content) in 95 per cent ethyl alcohol with 80 cc of 1 per cent ammonium oxalate. This is more permanent than the anilin gentian violet reagent.

Gram's iodine solution In a mortar grind up 1 gram of iodine with 2 grams of potassium iodide, when powdered, add distilled water a little at a time, triturating with the pestle. Pour into a bottle and rinse the mortar, using a total of 300 cc of water. It may also be prepared by diluting 5 cc of Lugol's solution with 70 cc of water.

Safranin Mix 10 cc of a 2.5 per cent solution in 95 per cent alcohol with 90 cc of water.

(c) *Ziehl-Neelsen Stain for Tubercle Bacilli*—Cover the fixed smear with carbolfuchsin and warm it sufficiently to keep the solution steaming for 3 to 5 minutes. Add stain as necessary to prevent drying. Since drying ruins the result it is still better to immerse the slides in the carbolfuchsin in a staining jar for 30 minutes or longer at room temperature.

Wash in water and decolorize in acid alcohol until the stain ceases to be dissolved from any but thick parts of the smear. Wash in water and counterstain 5 to 30 seconds with Loeffler's methylene blue. Allow to dry in the air.

Tubercle bacilli and other acid fast organisms (smegma bacillus, lepra bacillus) appear red, all else in a satisfactory portion of the smear is stained blue. Care should be taken not to confuse scratch marks in the slide with these organisms. Their appearance can best be learned by staining a smear of blood serum on a badly scratched slide and comparing with a stained smear of sputum containing many tubercle bacilli made on a new slide. Scratched slides should, of course, not be used for this purpose. See page 515 for a method of concentrating tubercle bacilli.

Reagents Czapelewsky's carbolfuchsin. Add to 1 gram of basic fuchsin, 5 cc of phenol liquefied by warming the can or bottle in a bath of hot water, and 50 cc

of glycerol, stirring constantly. Then add 50 cc of water, mix thoroughly and filter. This keeps indefinitely.

Acid alcohol. Add 5 cc of C P hydrochloric acid to 100 cc of 95 per cent ethyl alcohol.

3 Obtaining Material for Culture—The organism sought should always be indicated and the material kept in the ice box if available and otherwise at room temperature until inoculation on the correct medium has been made.

(a) *Blood Culture*—Sterilize the arm thoroughly with tincture of iodine and then alcohol. Withdraw blood by venipuncture using a sterile syringe and needle and after flaming the mouth of the flask, introduce 10 cc not less than 5 cc into 200 to 250 cc of dextrose infusion broth medium, preferably containing calcium carbonate. It is still better to introduce 9 cc of blood into a vaccine vial containing 1 or 2 cc of sterile 3.5 per cent sodium citrate solution, mix thoroughly and send to the laboratory for pour plates and inoculation into media suitable for the growth of the organisms sought. The use of a syringe and needle and inoculation through rubber caps greatly reduces the incidence of contamination when cultures are taken by persons who are not expert bacteriologists.

(b) *Culture for Diphtheria*—Roll a sterile cotton swab lightly over the involved membrane and streak on the surface of a slant of tellurite agar or Loeffler's medium leaving the swab in the tube. See that the cotton plug fits tightly around the shaft of the swab.

(c) *Other Cultures*—Spinal fluid and fluid from joints, the pleural cavity, the peritoneum, from an unopened abscess or boil and any other infected material that can be obtained readily by needle puncture is best sent to the laboratory in vaccine vials in citrate solution as described for blood culture. Urine should be obtained by catheter with a aseptic technic and sent to the laboratory in a sterile container. Material for stool culture is best obtained through a proctoscope but may be passed into a sterile container. Nearly all other cultures can best be sent to the laboratory on a blood agar slant with the swab accompanying as directed under cultures for diphtheria.

4 Pneumococcus Typing (Neufeld)—Material for typing is best obtained before administration of sulfapyridine. To a loopful or two of sputum, spinal fluid or pleural exudate on a slide add an equal volume of typing serum and of a 1-5 dilution of Loeffler's methylene blue. Mix and drop a vaseline rimmed cover glass over the mount and seal the edges by gentle pressure. Examine under the oil immersion lens after 5 minutes. A positive reaction is characterized by distinct swelling of the capsules which are unstained and by a sharp outline making the capsules readily distinguishable. If many organisms are present in the field make a fresh mount using a smaller amount of material. Not more than 3 or 4 diplococci should be present in one field. Test with each of the group serums labeled A, B, C etc., first and then with each of the specific type serums included in the group giving a positive reaction. Satisfactory typing serums are obtainable from Lederle Laboratories Inc., New York, and full directions and illustrations accompany the serums. The test may also be done on material obtained from the peritoneal cavity of the mouse 3 or 4 hours after intraperitoneal inoculation. This method should be used if pneumococci are not found by direct smear.

5 Examination for Treponema Pallidum—Scrape the surface of the lesion until a little clear serum is expressed. Make a moist cover slip preparation (page

497) for darkfield examination (page 332) for motile, tightly coiled spirals. If the material is to be sent to a central laboratory, collect a drop of serum by capillary in a length of capillary tubing and seal both ends in a flame.

B Serologic Methods — 1 Blood Typing — This is an absolutely essential preliminary to blood transfusion, since the intravenous injection of blood, the red cells of which are agglutinated by the serum of the recipient, leads to serious and often fatal reactions.

(a) *Principle* — Serum and cells of human blood can be grouped into four or more classes on the basis of their agglutinating properties.

(b) *Direct Matching* — This should be done before transfusion, whether the donor has been previously selected by determination of his blood group or not.

Withdraw 1 to 3 cc of blood from the vein of the recipient by the usual technique. Introduce 1 drop, or 2 drops if very anemic, into a tube containing about 1 cc of 1 per cent sodium citrate dissolved in 0.85 per cent sodium chloride solution, mix label it as the red cell suspension of the recipient, and introduce the remainder into a dry centrifuge tube, which is labelled recipient's serum.¹ The centrifuge tube should be sterile if it is to be kept more than a few hours. The patient's name and the date and hour of drawing the blood should also be noted on the label. Secure serum and cell suspension in a similar manner from the donor, if previously selected by blood grouping, or from a series of prospective donors if the blood types have not been determined. The serum may be expressed more rapidly from the clot by centrifugation.

With a large platinum loop or, better, a freshly made capillary pipette (use a different pipette for each serum and cell suspension) mix two parts of recipient's serum with one part of donor's cell suspension to form a large drop on a slide, and label it with a wax pencil R S D C. Mix in a similar manner two parts of the donor's serum and one part of the recipient's cells. Mount and label D S R C. To prevent drying keep the slides when not being examined resting on applicator sticks in a covered petri dish in the bottom of which is a piece of moist blotting or filter paper. Examine under the low power or, better, the 8 mm objective of a microscope at intervals during a 30 minute period, each time after the completion of the examination tipping the slide in rotary manner in order to produce a mixing motion in the drop. If at the end of 30 minutes no clumping of cells has occurred in either preparation the bloods are compatible. If at any time clumping occurs in R S D C or in both drops, the bloods are incompatible and another donor must be sought. If clumping occurs only in D S R C, transfusion will probably not cause violent reactions and in emergencies is permissible. If no emergency exists, it is better to secure a different donor. The clumping in true agglutination is into relatively large masses usually visible with the naked eye, which are not separated by rotary mixing. Sticking together of 1 or 2 cells or rouleaux formation (piling up like a stack of coins) is not significant. If there is any doubt as to the agglutination, it is safer to secure a different donor.

(c) *Determination of Blood Group* — If bloods are compatible to direct matching transfusion is safe as regards dangers of reaction from incompatibility, but direct matching often involves study of a large number of individuals before compatible blood is found. Hence if much typing is to be done, preliminary determination of the blood group of the recipient and of the prospective donors saves time and

¹ See Weiner reference page 276

² Cells and plasma from oxalated blood are equally satisfactory

serves as an additional check. Stock preparations of known type A(II) and type B(III) serums are necessary, these may be secured from biologic supply houses. A better plan is to type individuals working in the laboratory. Blood serum from such as are in type A and type B is secured from time to time to replenish the supply. If handled aseptically and kept in the ice box serum will remain usable a long time but it is usually more convenient to dilute it with an equal volume of glycerin, which obviates the necessity of aseptic technic. Or the serum may be dried and redissolved in 0.85 per cent salt solution from time to time. Any serum used for typing should be tested against a number of known cell suspensions to be sure it does not contain atypical agglutinins¹ and agglutinates the proper cells promptly in a dilution of at least 1 to 8. Great care should be exercised to prevent any mix up between the two. Always look at the label twice before making a mount to be certain that the right serum is being used.

Secure cell suspensions in the saline citrate solution by finger or ear puncture or by venipuncture from the recipient and the prospective donors. Make two mounts from each cell suspension according to the technic given above: one with known type A, the other with known type B serum and observe for agglutination as in direct matching.

The blood group to which the individual belongs is readily determinable from Table 46. This is the Landsteiner classification and the one that should be used. In the Moss and Jansky classifications groups II and III correspond to Landsteiner groups A and B, respectively. Group AB corresponds to group I Moss and group IV Jansky and group O corresponds to group IV Moss and group I Jansky. It is very important to note the classification.

TABLE 46—DETERMINATION OF BLOOD GROUPS

Cells	Serum			
	AB	A	B	O
AB	o	X	X	X
A	o	o	X	X
B	o	X	o	X
O	o	o	o	o

X = agglutination o = no agglutination

Individuals in group AB are sometimes called universal recipients and in great emergencies such individuals may be transfused with untyped blood.

Individuals in group O are sometimes called universal donors and in emergencies it is permissible to use blood from such a donor to transfuse an untyped recipient. In either case the recipient's serum should be matched with the donor's cells.

Except in such emergencies a donor of the same group as the recipient should be selected and direct matching performed as a further check.

Every person typed should receive a written statement showing the blood group in which he belongs and the classification used and should be instructed as to the importance of giving this information to any physician who may see him in an emergency. Large hospitals and clinics should keep a list of a number of persons

¹ Wilhelm M. M. and Osgood E. E. An Unusual Blood Group. Arch. Int. Med. 52: 133-136 (July) 1933.

willing to be donors, whose blood groups have been determined. Then, when an emergency arises, only the recipient need be typed and a donor in the correct blood group can be called at once for direct matching.

2 **Securing Blood for Serologic Tests** (Kolmer, Kahn, Kline, Widal, Brucella abortus agglutination, etc.)—Blood should be drawn from the vein in the usual way, using a *dry* sterile needle and a *dry* syringe, preferably, but not necessarily, sterile, *after removing the needle*, it should be introduced into a small dry, preferably sterile container such as a test tube or small vial, and corked. It is possible to perform any one of these tests on serum from as little as 1 cc. of blood, but chances of error are far less if 5 cc. are sent.

3 **The Donath-Landsteiner Test for Paroxysmal Hemoglobinuria**—(a) *Principle*—The blood of patients with this condition contains an iso- and auto hemolysin which unites with red cells only at low temperatures (2° to 10° , rarely as high as 18° C) and results in hemolysis only after warming to 37° C and in the presence of complement. The test is designed to demonstrate these points.

(b) *Technic*—Prepare a suspension of red cells not necessarily the patient's, and collect a sample of serum from the patient as directed under blood matching above. Mix equal parts of serum and cells and divide into two equal portions. Keep one at room temperature (20° or over) for the control. Chill the other in ice water or an ice box to 2° for 7 minutes. Compare the two tubes, there should be no difference. Place both tubes in the incubator or a bath at 37° . If the test is positive, definite hemolysis will have occurred in the chilled tube within 30 minutes (usually less) and not in the control. If no hemolysis has occurred in this time, add one fourth volume of normal serum to supply a possible deficiency of complement to each tube and incubate another 30 minutes. A positive hemolysis in tube I and none in tube II indicates the presence of the hemolysin characteristic of this disease, absence of hemolysis in both tubes indicates no such hemolysin and presence of hemolysis in both tubes indicates a flaw in technic.

The above is all that is necessary for diagnostic purposes. It is of academic interest to run a series of tubes cooling to different temperatures to determine the highest temperature at which hemolysis occurs. Further details and bibliography will be found in the review by Mackenzie cited on p. 22.

4 **The Paul and Bunnell Test¹ for Infectious Mononucleosis**—This test should be done whenever prolymphocytes are found in the differential cell count.

(a) *Technic*—Inactivate the serum for 15 minutes at 56° . Make a series of dilutions of the serum with 0.9 per cent saline of from 1 to 4, 1 to 8, etc., up to 1 to 256, leaving 0.5 cc. of each dilution in $4 \times \frac{1}{2}$ in test tubes. To each tube add 0.5 cc. of fresh 2 per cent suspension of washed sheep erythrocytes and 1 cc. of 0.9 per cent saline, mix, place in a water bath at 37.5° for 1 hour and allow to stand in the ice box over night. After inverting the tubes 3 times, read according to the dilution and the degree of agglutination, 1 plus being barely perceptible agglutination and 4 plus, a firm disk. An agglutination occurring in a dilution higher than 1 to 32 is diagnostic of infectious mononucleosis or serum disease.

Davidsohn² has described a test which will differentiate the agglutinins of infectious mononucleosis from those which occur in serum disease.

¹ Paul J. R. and Bunnell W. W. Presence of Heterophile Antibodies in Infectious Mononucleosis. *Am. J. M. Sci.* 183: 90 (Jan.) 1932.

Bunnell W. W. Diagnostic Test for Infectious Mononucleosis. *Am. J. M. Sci.* 186: 346 (Sept.) 1933.

² See reference on page 261.

SECTION XI SPUTUM EXAMINATION

Sputum always contains bacteria and usually virulent organisms, hence it must be handled with care

A Collection and Labelling of Specimens—1 **Containers**—Wide mouth glass bottles are preferable. Two sizes are desirable, a smaller one of 3 to 4 ounce capacity for routine use and a larger one of 8 ounce capacity for use when large amounts are expected. Urine specimen bottles are satisfactory. Paper containers have the advantage of being easily destroyed but are unsatisfactory for volume determination and inspection.

2 **Care of Glassware**—The containers previously emptied into lysol or incinerator, slides and glass plates should be boiled or autoclaved in 1 or 2 per cent dilution of liquor cresolis compositus or lysol washed free of antiseptic in running tap water, and immersed for 24 hours in cleaning fluid (1 part of 20 per cent potassium bichromate¹ solution to which is added *slowly* 2 parts of concentrated sulphuric acid²). They should then be rinsed in tap water and dried preferably in a dry sterilizer at 220° C. The containers should be stored upside down and the slides and glass plates should be kept protected from dust.

3 **Directions to the Patient**—These have been given on p. 280. The patient should be warned not to contaminate the exterior of the bottle. A further precaution that is desirable is to fasten a piece of paper towel about the container with rubber bands during the collection of the specimen.

4 **Labelling and Transportation to the Laboratory**—The label should be substituted for the paper towel which should be burned before the specimen is sent to the laboratory. It should show the hours of beginning and ending the collection preferably a 24 hour period beginning at 8 A. M. as well as the name, date, tentative diagnosis and tests desired. If the sputum is to be shipped the bottle should be tightly stoppered with a new paraffin coated cork and sent in a cardboard container. If it is to be sent to a laboratory in the same building where collected, a piece of paper towel fastened over the mouth of the bottle with a rubber band is best. Corks which have been used in sputum bottles should be sterilized by autoclaving before they are used again or better discarded for incineration.

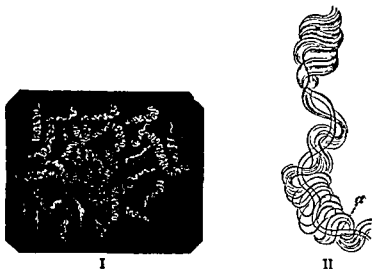
B Gross Examination—1 **Note the Volume**. This is best done by comparison with a similar container on the side of which a scale graduated in 10 cc. intervals has been marked with a file as this is sufficiently accurate for clinical purposes and avoids the danger of contamination and the unpleasantness of a transfer to a measuring cylinder.

¹ These keep indefinitely when separated but the mixture should be made fresh once in two weeks.

2 **Note the Appearance and Consistency**—Describe it in such terms as mucoid, mucopurulent, purulent, serous, bloody, watery, or frothy. Note whether it separates into three layers on standing if the volume is over 25 cc. Note any caseous (cheesy) masses which may be present and select these for direct smears for tubercle bacilli.

3 **Describe the Color**—If green, test for bile pigment and culture for *Pseudomonas aeruginosa* (*B. pyocyaneus*).

Blood in the sputum (hemoptysis) differs from vomited blood (hematemesis) in being bright red and frothy and alkaline in reaction,



I

II

FIG 27—Curschmann's spirals (Hobster after Curschmann) I, Natural size II enlarged a central thread

whereas vomited blood is usually brownish and acid and never frothy. Describe the blood present in such terms as blood streaked, small clots, blood unmixed with sputum, "rusty," "prune juice appearance" and indicate the amount on a scale of 1 to 4 plus.

4 **Describe the Odor**—It may be foul (1 to 4 plus), putrefactive, sweetish, or odorless.

5 **Spread the sputum between glass plates and look for Curschmann's spirals, elastic tissue, Ditttrich's plugs, bronchial casts, and purulent or caseous masses.** Wooden applicators or tongue blades which can be discarded for incineration are most satisfactory for transferring the sputum to the plates. Curschmann's spirals are spiral coils of mucus (see Fig 27). Elastic tissue which is identified by the uniform diameter and branching of the fibers is very important. Ditttrich's plugs are sausage shaped casts of the bronchi, varying in size up to that of a white bean, seen frequently in bronchial asthma.

Bronchial casts have the shape of the portion of the bronchial tree from which they came

C Microscopic Examination—1 **Unstained**—Anything suspicious noted on gross examination between the glass plates should be further studied under low power and the identification thus be completed. High power may be necessary to identify the colorless or yellowish, sharp pointed octahedra called Charcot Leyden crystals which are derived from the disintegration of eosinophils. Elastic tissue is best detected by boiling the sputum with a double volume of 10 per cent sodium hydroxide and examination of the centrifugated sediment with the low power. Rarely the brownish oval ova of *Paragonimus westermani* may be found. They are operculated and measure 60 by 90 micra.

2 **Stained**—(a) *Wright's Stain*—Make a thin smear and use the same technic as for blood smears in staining.

If bronchial asthma is suspected, a smear of sputum should always be stained with Wright's stain and examined under the oil immersion for eosinophils.

Large numbers of neutrophils suggest pyogenic infections or, if heavily laden with pigment granules (heart failure cells) they indicate chronic passive congestion.

The presence of small lymphocytes, monocytes and epithelial cells should also be noted.

(b) *Methylthionine chloride (Methylene Blue) and Gram's Stains*—These should be done routinely. The technic has been given. Record the predominant organism. Look especially for organisms having the staining characteristics of pneumococci, streptococci and Vincent's organisms. Describe any others present in numbers.

(c) *Examination for Tubercle Bacilli*—The Ziehl-Neelsen stain has been described. It should be done on a direct smear of all sputum specimens examined. If the direct smear is negative in cases in which tuberculosis enters into the differential diagnosis a concentration technic should be employed. Matson's modification of the Hermann and Ilandsen technic is recommended.

To 1 part of sputum (10 or 15 cc) add one part of 0.6 per cent sodium carbonate. Place in a stoppered bottle. Shake well and incubate 24 hours at 37° C. Centrifuge at high speed for 15 minutes. Pour off the supernatant fluid. Mix the sediment with an equal volume of 30 per cent antiformin. Allow the sediment to stand 10 to 20

¹Matson, F. C. Der Vergleichswert einer neueren Methoden der Sputumuntersuchung auf Tuberkulbazillen des Ziehl-Neelsen und Methylthionin Typus. Beitr. z. Klin. d. Tuberk. 24: 193-215, 1912.

ing to whether the albumin test is one plus or four plus. The result must, of course, be multiplied by the dilution factor and divided by 1000 to change it from milligrams to grams per 100 cc.

(c) *Other Quantitative Tests*—These are not part of the routine examination of the spinal fluid, but are often of value in cases of coma, when a spinal puncture is being done anyway and it is desired to learn at the earliest possible moment whether or not a patient has uremic or diabetic coma. A chloride estimation is desirable in cases of possible tuberculous meningitis.

Emergency Tests for Quick Detection of Uremia (see methods 1 to 4 below) —

1 *Partial Non-protein Nitrogen Estimation*—This is an application of the salivary urea method of Hench and Aldrich.

Technic Measure exactly 1 cc of clear spinal fluid into a beaker, add exactly 3 cc of 2 per cent mercuric chloride solution. Mix and remove a drop and test it with 8.5 per cent sodium carbonate solution as in the salivary urea method. If a positive test is secured, there is not much retention (no uremia). If negative, continue adding the bichloride solution, 0.5 cc. at a time, testing after each addition until a brown precipitate appears. A titration of 3.1 cc was obtained when spinal fluid contained 46 mg of non protein nitrogen per 100 cc, and a titration of 4.0 cc when it contained 60 mg.

If the spinal fluid contains blood or protein, treat 2 cc of it with 0.02 cc or 0.04 cc, according to the amount of protein present, of 10 per cent trichloroacetic acid solution and centrifugate. Measure 1 cc of the clear fluid into a beaker, treat it with 0.2 cc or 0.4 cc, according to the amount of trichloroacetic acid used of *M/10 disodium phosphate* (1.42 gm of anhydrous phosphate in 100 cc) and titrate. To compensate for the error caused by the presence of trichloroacetic acid, add 3.3 cc, if the smaller amount of acid was used or 3.6 cc, if the larger amount of bichloride at the start but note it down as only 3 cc.

2 *Quick Approximate Estimation of Creatinine*—To 1 cc of clear spinal fluid add 9 cc of distilled water and 5 cc of picrate reagent. Mix well and pour into a half ounce wide mouth square bottle. After 8 minutes compare with the permanent dichromate standards which correspond to 3, 4, 5 or 6 mg of creatinine per 100 cc of spinal fluid, using plain white paper as a background and having the light pass over the shoulder directly to the bottles not from one side. The 'daylight' electric lamp may be used if sunlight is not available.

Reagents Picrate reagent. Mix 5 cc of saturated picric acid solution with 1 cc of clear 10 per cent sodium hydroxide just before using.

Saturated picric acid This is about 1.1 per cent. No heat must be used in dissolving it. Use only the very best picric acid. Baker's C.P. "special for blood tests" is very satisfactory. Keep the solution in a brown bottle. Make it fresh once a month. The discarded solution can be used for blood creatinine estimations.

The standards Make these from *N/10 potassium dichromate* (0.491 gram per 100 cc) solution by the following dilutions and keep them in half ounce square bottles tightly corked.

3 mg standard 5 cc dichromate plus 8.5 cc water

4 mg standard 5 cc dichromate plus 4.0 cc water

5 mg standard 5 cc dichromate plus 2.3 cc water

6 mg standard 5 cc dichromate plus 2.0 cc water

Keep the bottles away from the light when not in use.

3 Estimation of Creatinine, Using a Colorimeter—Dilute 1 cc of spinal fluid with 9 cc of distilled water. Treat this exactly as if it were 10 cc of protein free blood filtrate. The technic and calculation are exactly the same as for blood creatinine estimation (page 387). It is also well to prepare a creatinine standard of double strength (see blood creatinine method) if this is used substitute 3 for 15 in the formula for calculation.

4 Estimation of Urea Nitrogen—Measure exactly 1 cc of the spinal fluid into tube 2 of the aeration apparatus, add buffer and urease solutions and continue exactly as in the blood urea nitrogen method (page 381).

Calculation Deduct the control for urease from the amount of N/70 acid that was neutralized by ammonia and multiply by 20. The result is the mg of urea nitrogen per 100 cc of spinal fluid.

Emergency Tests for Quick Detection of Diabetic Acidosis (see methods 5 and 6 below)—5 Estimation of the Alkali Reserve—Measure 2 cc of clear spinal fluid treat it with 5 cc of N/50 hydrochloric acid and titrate with N/50 sodium hydroxide exactly as if plasma were being used. If there is very little turbidity use the clear standard. The calculation is the same as for plasma (page 396).

6 Detection of Marked Hyperglycemia.—Measure exactly 0.5 cc of spinal fluid and 4.5 cc of water into a large test tube, add 5 cc of Shaffer Hartman micro copper reagent and continue the estimation exactly as if 5 cc of protein free blood filtrate were being used (page 373).

Calculation Find the per cent of dextrose in the spinal fluid in column B of the table opposite the cc of thiosulphate used for titration.

7 Chloride Estimation—To exactly 1 cc of cerebrospinal fluid add exactly 9 cc of distilled water. Proceed with this just as directed for blood filtrate (page 395). The calculation is also the same. Report the results as mg of sodium chloride per 100 cc of fluid.

See Chapter X and Table 16 (page 291) for normal values for all cerebrospinal fluid results.

8 Serologic Tests—Reserve 5 cc, not less than 3 cc, of cerebrospinal fluid in a sterile container for the Kolmer Kahn and Lange tests. It is desirable to have these tests done on all fluids examined and they are specifically indicated in any case in which any form of syphilis of the nervous system is considered in the diagnosis.

(a) *Lange Test*—(1) Principle—Equal quantities of colloidal gold solution are added to 10 test tubes containing dilutions of spinal fluid 1 to 10, 1 to 20, 1 to 40, etc., up to 1 to 5120. After standing 24 hours the tubes are read from the lowest dilution on the left to the highest dilution on the right. The amount of precipitation and the dilution in which it occurs are related to the globulin content of the fluid but the exact mechanism is unknown.

(2) Technic—Into the first of 10 clean dry test tubes reserved especially for the purpose, put 0.9 cc of fresh 0.4 per cent sodium chloride solution. Into each of the remaining 9 tubes put 0.5 cc of 0.4 per cent salt solution. Now add to the first tube 0.1 cc of the spinal fluid to be tested. Mix well. Transfer 0.5 cc to the next tube mix, transfer 0.5 cc to the third tube. Proceed in this manner up to and including the tenth tube. Discard the 0.5 cc from the last tube. To the eleventh tube add 0.5 cc of the 0.4 per cent salt solution this is the control tube. To all tubes now add 2.5 cc of the colloidal gold solution. Shake each tube thoroughly. Read the tubes after they have stood over night at room temperature.

All readings must be done with direct daylight, holding the tubes up against the sky. The amount of color change, due to precipitation, in the colloidal gold solution is expressed numerically. Thus, 0 means no change, 1 means very slight change, 2 means reddish violet, 3 means violet, 4 means blue, 5 means colorless, complete precipitation.

(3) Reagents and Glassware —All glassware used should be Pyrex, cleaned with aqua regia (3 parts of C P hydrochloric acid and 1 part of C P nitric acid), and rinsed repeatedly with triple distilled water, dried and kept protected from dust after use.

Preparation of colloidal gold All of the reagents should be the purest available (Merck's blue label are satisfactory) and all water should be freshly boiled distilled water, preferably triple distilled from a still having no rubber connections. When ready to start the preparation, have available a large water bath, a liter of freshly boiled distilled water, a 1 per cent solution of gold chloride, a freshly prepared 2 per cent potassium carbonate solution, a 1 per cent solution of formaldehyde (2.2 cc of 40 per cent diluted to 100 cc), and a clean dry 100° thermometer. Prepare a small trial lot first. Heat 50 cc of the distilled water in the water bath until the thermometer in the distilled water registers 60°. Add 0.5 cc of 1 per cent gold chloride, shake, and add 0.5 cc of 2 per cent potassium carbonate, shake, and return to the bath. Heat to 90-92°, turn out the flame, add 1 per cent formalin drop by drop with shaking until the red color appears. Add 1 or 2 drops more. The solution should be a brilliant reddish orange with no trace of blue. If it is bluish, repeat using less of the potassium carbonate solution. When the right combination is found, make a large batch, multiplying by 10 or 20. Test the gold after cooling with a known negative and a known paretic spinal fluid.

Normal spinal fluids show no change. Read 0000000000.

B Examination of Exudates, Transudates, Secretions and Cyst Fluids —The specific gravity, protein content, and cell count should be determined on all fluids which are not grossly purulent. The bacterial stains and cultures should be done on all fluids which prove to be exudates and on all grossly purulent specimens. A differential cell count should be done on all fluids showing a cell count above 100 per c mm. Do the other tests as ordered or indicated. The technic is essentially the same whether the fluid be derived from the pleural, pericardial, peritoneal, or joint cavities or from a cyst or abscess. Since fluids occasionally clot, it is safer to place 10 cc in a test tube containing 20 mg of potassium oxalate, as for blood, to be used for the white and differential cell counts. The fluid must, of course, be collected in a sterile container with aseptic precautions, if cultures are desired.

1 **Gross Examination** —Note the total volume removed, describe the color and appearance. Record the specific gravity.

2 **Protein Content** —(a) *Total Protein Content* —Determine this by performing an Esbach test on a 1 to 10 dilution of the fluid and

multiplying the result by 10. If only a faint cloud appears, empty the tube and perform the test on the undiluted fluid (page 360). It is better to use the method given for quantitative estimation of protein in cerebrospinal fluid on a 1 to 100 dilution in 0.9 per cent sodium chloride solution (page 519).

(b) *Rivalta Test*—Add 0.2 cc. of 10 per cent acetic acid to 100 cc. of distilled water in a tall cylinder. Mix, and add a drop of the fluid to be tested. If the fluid is an exudate, a white cloud appears as the drop sinks in the liquid. Transudates give only a very faint cloud or none at all. This test detects the differences in globulin content.

3 **Microscopic Examination**—(a) *Cell Count*—If not grossly purulent or bloody, do a white cell count by the technic used for blood if a high count is expected or by the technic for spinal fluid if a low count is expected. The cell count should be done within 1 hour of the time of withdrawing the fluid.

(b) *Differential Cell Count and Bacteriologic Examination*—Centrifuge a 10 cc. portion of the fluid if cells are scarce and make smears with the sediment in the usual way. If cells are very numerous, direct smears will suffice. Wright's and Gram's stains are to be made as a routine. Do a differential count on the Wright's stained smear and look for bacteria in both preparations. Note whether red cells are present or absent.

For the detection of tubercle bacilli, inoculation of a guinea pig in the inguinal region with 10 cc. of fluid secured under sterile conditions is usually necessary. Occasionally, however, tubercle bacilli may be demonstrated by treating the sediment from a large volume of fluid with an equal volume of 30 per cent antiformin, diluting, centrifuging, making smears with the concentrate, and staining by the Ziehl-Neelsen method.

(c) *Tumor cells*¹ may in rare instances be demonstrated in effusions resulting from certain types of malignant disease.

(d) *In cyst fluids* look also for hooklets of *Taenia echinococcus* (rare).

4 **Quantitative Chemical Examination**—This is rarely indicated. Use the directions given for the preparation of Folin filtrate from plasma and the methods given in the section on blood chemistry. Exactly the same technic as for blood is satisfactory for urea nitrogen determinations.

¹Foot N. C. The Identification of Tumor Cells in Sediments of Serous Effusions. *Am. J. Path.* 13: 1-12 (Jan.) 1937.

SECTION XIII MISCELLANEOUS METHODS

A Vital Capacity Determination—The patient, preferably in the standing or sitting position, is instructed to take the deepest possible inspiration and then empty his lungs as completely as possible into a spirometer. The spirometer of the McKesson or Benedict basal metabolism machines may be used. The average volume of air expired on several trials is the total vital capacity. No corrections are made for temperature and pressure. From the patient's height and weight, find the surface area from Table 25 (p. 426) and report the result as liters per square meter of body surface.

B Examination of Genital Secretions—1 **Prostatic Fluid**.—The fluid, obtained by prostatic massage immediately after urination, should be examined grossly between glass plates as described for sputum and microscopically both unstained and with methylene blue and Gram's stains. The fluid is normally grayish white, tenacious and turbid and sometimes contains semi-solid clumps. Microscopically, spermin crystals (four-sided prisms) and spermatozoa (Fig. 11) which may be either active or dead are normal constituents.

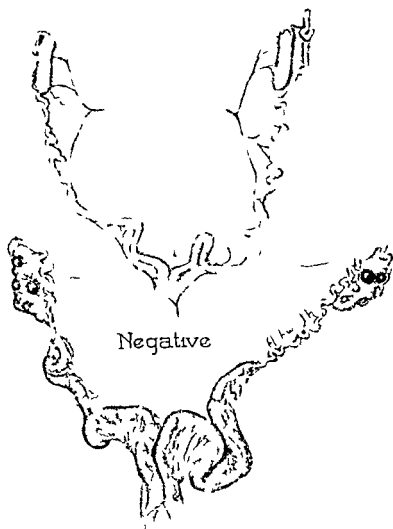
The most important deviations from the normal to record are a grossly purulent or bloody appearance and the character and approximate quantities of any pus cells or bacteria if found on microscopic examination. Of the latter, the gonococcus is most important. This is a gram-negative biscuit-shaped diplococcus which is often intracellular.

2 **Semen**—This is usually studied as an aid in determining the cause of sterility. Hence, the chief point to observe is the number of spermatozoa present and their activity. They normally progress rapidly across the field of a moist cover slip preparation with a violent whip-like motion of their tails. Only in inactive forms can the morphology (see Fig. 11) be well seen. Other points to note are the same as in examination of prostatic fluid.

The specimen should be examined as soon as possible after intercourse (within one hour). It is desirable to have the woman come as soon as possible after intercourse to the place where the examination is to be made and the physician should there collect a specimen from the vaginal vault and from the cervical canal. If these show numerous active spermatozoa, no other study is necessary, if they do not, a condom specimen or better a specimen collected manually in the office in a glass container is desirable in addition.

Note the volume, describe the gross appearance, and examine a moist cover slip preparation microscopically. Record the approximate number of spermatozoa present, the percentage of actively motile forms and the presence of pus or red cells. A spermatozoa count¹ may be made by diluting 0.5 cc. of well-mixed semen with 19.5 cc. of 5 per cent sodium bicarbonate solution in 1 per cent formalin, mixing thoroughly, mounting on a hemocytometer counting chamber and counting the

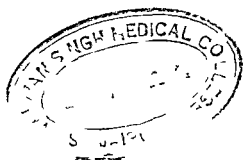
¹ Belding, D. L. Fertility in the Male. I. Technical Problems in Establishing Standards of Fertility. *Am. J. Obst. and Gynec.* 26: 868 (Dec.) 1933. II. Technic of the Spermatozoa Count. *Am. J. Obst. and Gynec.* 27: 25 (Jan.) 1934.



Positive

PLATE IX.—The Friedman pregnancy test

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injection Aseptic precautions are not necessary Kill the rabbit 36, or preferably 48, hours later by a blow on the back of the neck Open the abdominal cavity and examine the tubes and ovaries A positive test is indicated by the appearance of the reddish, hemorrhagic corpora lutea, illustrated in Plate IV In a negative test, the tubes and ovaries are much smaller and show no hemorrhagic corpora lutea The accuracy of the test is slightly greater if 2 rabbits are used An extremely positive test in which the whole ovary is a mass of hemorrhagic nodules suggests a *chorione epithelioma* and repetition of the test, using only 1 cc. of urine

Many variations of this technic have been suggested but the method outlined above has proved more satisfactory and economical It is absolutely essential that the rabbits be kept in separate pens if the results are to be dependable

If a negative test is secured on urine voided less than 10 days after the date when the first missed menstrual period should have begun the test should be repeated on urine voided after this date before using the negative result as evidence against the diagnosis of pregnancy A positive result in the first 10 days is significant and sometimes occurs

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INDEX BY DISEASES

Plan of the Index

In this index I have arranged alphabetically most of the diseases and syndromes in which laboratory methods are of material aid. Following each disease is the page reference to the chief discussion of that disease when such a discussion is given. Diseases or syndromes which are common and in which laboratory study is of major importance are given in boldface type. Following the page reference in many instances is a list of conditions which should also be consulted in this index. These conditions include common complications, diseases which may simulate the one under consideration, etc.

Under each disease or syndrome are listed the laboratory procedures and findings which may be of value or interest in the study of this condition. These are grouped as follows. Under A are listed those procedures which an intern in a modern hospital could reasonably be expected to order within twenty four hours after the admission of a patient presenting this syndrome, or in whom this disease was considered in the differential diagnosis, in other words, the tests that should be done on all these cases. Under B are listed the additional tests which are of value in early, acute or especially puzzling cases. Under C are listed the additional tests which are of value in late, chronic or especially severe cases. Under D are listed tests which may give deviations from the normal in this condition, but which are so difficult or so dangerous that they would be performed only in research studies, or which give results that are of academic interest rather than of definite value in making the diagnosis or in caring for the patient. After each test is given in ordinary type the page references to the discussion of the interpretation of that test and in boldface type, page references to the technic for that test. Absence of a reference in either kind of type means that the corresponding discussion will not be found in this book and should be sought in reference works on the particular subject. This applies to most of the bacteriologic and serologic procedures mentioned. An effort has been made to use terminology which will suggest not only the tests to perform, but also the deviation from the normal which may occur in that condition. To save repeti-

tion, common syndromes such as acidosis, anemia, diarrhea, jaundice, vomiting, etc. which always demand a certain group of laboratory studies are listed alphabetically as if they were diseases and mention of the syndrome with *q v* following it is given after the diseases in which it occurs. Procedures such as the routine urinalysis, hematologic examination, serologic tests for syphilis, and sedimentation rate *which should be done on every patient* are listed only when the results are of particular value or interest. Procedures which are of great importance are given in italics. Tests which are of value in controlling treatment and in prognosis are given as well as those which are of diagnostic value.

Use of the Index

This index is not a substitute for cerebration, but *if used in accordance with the principles outlined in Chapter I* it should greatly aid the practitioner, intern, or student in planning rapidly and efficiently the laboratory study and in deriving the maximum information from the results obtained. After completion of the history or physical examination look up the diseases included in the differential diagnosis in the index, and any others which are suggested therein. Read the chief discussion if not already familiar with it. Glance down the list of procedures, ordering those indicated in the particular case, if the terminology used does not aid you to remember, look up the interpretation and technic. Bear in mind that only the usual deviations can be suggested in the index and that the whole discussion of the test must be understood to interpret the result properly. Hence, it will sometimes be necessary to read a page or more of interpretation before coming to the specific mention of the particular disease.

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- B Congo red test for amyloidosis 42 414
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B *Modified Mosenthal test*, 30 360

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A *Albuminuria* 14 342

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philia 237 238 240 242 476

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**D Non protein nitrogen increased 35
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- Poisoning radium or roentgen ray, 204 220
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NOTE TO THE INSTRUCTOR

There are no substitutes for the originality of presentation and the contagious enthusiasm of the *teacher* who loves his subject and loves his students. Nevertheless, since the division of the subject into two parts as in this book is a little unusual, a brief outline of my teaching technic may contain in it suggestions which will prove of value, and the appended schedule of assignments should simplify a correlation of the two parts. A rigid adherence to this schedule is not my desire. The order given is that which has proved most suitable in our institution where the course is given in the Spring term and the light for microscopic work is better toward the end of the season. A different order, putting the microscopic work first, might prove more suitable in schools where the course is given in the Fall term. In order to increase flexibility of arrangement, each chapter has been made fairly complete in itself even at the expense of some repetition which, however, is probably not without pedagogic value. Therefore, any order is possible. To increase flexibility of adaptation to the varied lengths of time devoted to this subject in different schools, less important points and more difficult or infrequently used methods have been put in fine print, and in the shorter courses such matter can be merely read or omitted entirely at the discretion of the instructor.

I devote the lecture time largely to quizzing over the assigned work, trying to phrase all questions in such a way that they will involve thinking, and emphasizing the practical clinical applications of the data by use of actual cases wherever possible. For example, I would outline the main points in the history of a case which is sufficiently clear cut to make it obvious to a sophomore student that it would belong in the group of conditions formerly included under the term Bright's disease and would ask that student to state what laboratory tests he would do or order. As each is mentioned I would give him the result of the test in the particular case and thus lead him eventually to arrive at the correct diagnosis. By requiring another student to state how he would perform the test the errors in diagnosis which might result from errors in technic can be emphasized. By selecting a few cases which are likely to come to post mortem and keeping the class in touch with the laboratory study of these cases, the value and limitations of laboratory study can be conclusively demonstrated. This method of teaching is applicable to almost every  of the subject. Care is

taken to spend most of the time on the more important points, to see that the correct answer to the question is ultimately so clearly stated that all ought surely to understand it, and to stress constantly the importance of thinking from the basic chemistry, physiology, and pathology of the disease to the laboratory changes and from these to the diagnosis. The resumé at the beginning of each chapter was planned to aid the student in this type of thinking and for the same reason more clinical medicine is included than is usual in texts on laboratory diagnosis. I believe no other subject better adapts itself to thus closing the gap between the fundamental sciences and clinical medicine.

The importance of adherence to the general principles outlined in Chapter I is illustrated at every opportunity, as are the various errors that may result from neglect of these principles. Any points that obviously have not been grasped from the study of the text are explained, and are quizzed over in subsequent sessions. New matter that has appeared since the last revision of the text and points of view differing from those expressed in the text may profitably be presented in lecture form as the corresponding subject is taken up. Toward the last of the course students are asked to hunt up a new method for some particular procedure and present it to the class. Then in seminar style the class is asked to point out advantages and disadvantages of the method. In a similar manner, articles on interpretation are discussed with the object of training the student in finding and critically evaluating new work in this subject. Definite effort is made to see that the students realize that they have only commenced the study of this subject and that its full value can only be appreciated after much experience in using it in the clinical years. While few instructors have the advantage possessed by the author of teaching physical diagnosis and differential diagnosis to the same students as Juniors and Seniors, arrangement with the clinical instructors can usually be made to secure cooperation in keeping awake the student's interest in the laboratory phase of medicine and in seeing that he keeps the laboratory examination in its proper place, i.e., subordinate and supplementary to the history and physical examination. I have found the Index by Diseases of great value in maintaining this interest, as well as an aid in reviewing the subject from a new angle at the end of the term.

In the laboratory, the student is introduced to each method by work on knowns until he has had opportunity thoroughly to satisfy himself of the proper outcome and has formed some opinion of the errors which may result from insufficient practice. He is then given

unknowns and graded on the proficiency displayed in the technic, the evidences of honesty in reporting the results, and the closeness with which he approaches the accuracy obtainable with the method used. The more difficult methods are demonstrated, but since the directions have been made extremely explicit and the assignments are intended to be studied before coming to class I feel that in most instances the student's time can be spent to better advantage in controlled practice as above outlined. The instructors in the laboratory seek to encourage the students to look upon them as friendly consultants to be called upon for suggestions when repeated trials still give erroneous results. Every opportunity is taken to stress the fact that if the results are to be clinically usable the method selected must not only be reliable, but it must also be performed with care, with controls on its accuracy and by someone who has had adequate practice. This is only possible when the correct result on all material used for class study is known to the instructor. More time is devoted to quantitative urine chemistry than its clinical importance justifies because the methods are so similar to the more important blood methods that facility acquired in the procedures for one is transferable to the other, and material sufficient for large classes is more easily secured. The laboratory periods should come on two or more successive days in the week in order to allow for completion of the longer methods and to prevent waste of material. The most satisfactory way of training students in the identification of casts, ova, abnormal red and white cells, etc. is to furnish the students with suitable material and to require each student to find and demonstrate a certain number of each type to the instructors before credit is given on this phase of the work.

For convenience detailed directions are given at the end of each method for the preparation of the more complex reagents and all are indexed under the term "Reagents" in the index. Reagents for which the preparation is obvious or for which directions have been given in other methods are italicized in the description of the technic. All reagents and standards mentioned in Part Two can be purchased from the Shaw Surgical Co., Portland, Oregon.

Before substituting other methods for those here indicated as preferred, which may sometimes prove desirable, I ask only one thing that is, that the two methods actually be tried out first in direct comparison with each other (and preferably with a research method, too) on known material under conditions of clinical use. This is the manner in which these methods have been chosen and it seems but just that they be discarded only on evidence of equal weight.

SCHEDULE OF ASSIGNMENTS*

Day	For quiz period		For laboratory period
	Part One	Part Two	
1	Prefaces 1-10	327-344(8) 359(B)-360(2) 519	Start Esbach test, 359(1) Check lockers Calibrate apparatus 327-330(E) Prepare standard solutions 336 337 Review protein tests on knowns 342(7)- 344(8)
2	11-22(3)	349(12)-356(19) 357(21)-359(B)	Read Esbach test and do new modifica- tion 359(1) Finish standard solutions 336 Estimate ammonia nitrogen and total nitrogen in known 24 hour urine 363(6)-367(8) Study known urine sedi- ments, 349(12)-356(19)
3	22(3)-32(1)	360(2)-371(9)	Read phenolsulphonphthalein tests 362(5) Do urea nitrogen on known 24 hour urine 365(7) Complete study of known sediments 349(12)-356(19) Start col- lection of dilution and concentration fractions 361(4)
4	32(F)-43(V)	371(9)-372(11) 381-383(D)	Determine volume and specific gravity 338-342(7), on dilution and concentra- tion fractions Qualitative protein 342(7)-344(8) and sediment unknowns 349(12)-356(19)
5	43(V)-54 Review Chapters I and II	385(D)-390(4) 414(B)-414(G)	Do urea clearance test 381-385(D) Prepare Folin filtrate from 12 cc of blood 383(D)-386() Add toluol to filtrate and save
6	55-69(VII)	344(8)-349(12) 405(4)-408(5)	Do blood creatinine on filtrate 387(2) and non protein nitrogen 386(E)-387(2) on blood filtrate Review qualitative sugar and ketosis tests on known urines 344(8)-349(12)
7	69(VII)-77(V)	372(11)-378(12) 390(4)-394(5)	Do blood urea nitrogen on unknown 381 Do plasma protein 405(4) 519
8	77(X)-93	394(5)-403(2) 378(13)-379(15)	Do qualitative and quantitative sugar estimations on knowns and unknowns 344(8)-349(12) 372(11)-378(12)
9	94-107(III)	4 3-433	Do alkali reserve 396(F) and blood sugar 385(D) 390(4) on knowns

* All figures and letters in parentheses refer to paragraphs All other figures refer to pages.

SCHEDULE OF ASSIGNMENTS *—(Continued)

Day	For quiz period		For laboratory period
10	107(III)-117 Review Chapters III and IV	403(2)-405(4) 408(5)-410(6)	Prepare filtrates from blood for blood sugar estimation 386(2) Add toluol and save Do blood sugar estimations on filtrate 390(4) Do blood calcium and phosphate determinations 403(2)-405(4)
11	118-133(III)	434-442	Begin basal metabolic rate determinations by groups 423-433 and continue daily until all groups have finished Do urea nitrogen on blood unknown 381 Do an alkali reserve 396(F) and blood chloride 395(7) on unknowns Do gas tric analyses on knowns 434-441
12	133(III)-145(IV)	356(19)-357(21) 378(12)-378(13) 410(6)-413(8)	Test qualitative and quantitative stomach contents unknowns 334-342 Do bile pigment bile salt and urobilinogen tests on own urine and knowns 356(19)-357(21) Demonstration of Boas Oppler bacilli and sarcinae
13	145(IV)-160	379(16)-380 443-447(E) Review Sections I II III	Do icterus index estimation 410(6) Demonstrate Van den Bergh test 412(b) One stomach contents unknown Two urine unknowns One blood unknown for urea nitrogen and dextrose
14	Review Chapters I II III IV and V	447(E)-457 Review Sections IV-VIII	Examine known feces samples 443-457 Examine qualitative and quantitative stomach contents unknowns Study and draw parasites and ova
15	Written examination over all covered to date		Do qualitative and quantitative urine unknowns 338-380 and alkali reserve unknown 396(F)
16	161-177(2)	458-462(5) 471(6)-476(8)	Practice red and white cell counts 471(6) Continue study of intestinal parasites and ova Practice making and staining of blood smears 476(8)
17	177(2)-188(V)	462(5)-471(6)	Continue red and white cell counts on knowns and own blood Make smears and hemoglobin estimations on known bloods 462(5) Continue study of intestinal parasites

All figures and letters in parentheses refer to paragraphs. All other figures refer to pages

SCHEDULE OF ASSIGNMENTS *—(Continued)

Day	For quiz period		For laboratory period
18	183(V)-202(V) Chart I, Plate II and legends	487(B)-493(7)	Continue work as above Turn in report of routine hematologic examination of own blood including color index 488(a)
19	202(V)-212(E)	476(8)-483(f)	Demonstrate cell volume determination Routine hematologic examination including calculation of color volume and saturation indexes on unknown bloods 487(B)-493(3)
20	212(E)-223(VI)	484(9)-487(B) 493(7)-501(10)	Study demonstrations of normal and abnormal red cells Do volume index color index and saturation index 487(B)-493(3) by groups on unknown bloods Begin study of anemia slides Do reticulocyte stain and count, 496(s)
21	223(VI)-227 Review Chapter VI	413(8)-414(G)	Continue study of unknown anemia slides, demonstrating each type of abnormal red cell to instructor Do erometer method of red cell diameter 493(4) Demonstration of sternal puncture 499(B)
22	228-240(C) Plates I, III IV and V with legends	475(7)-483(f)	Demonstrate red cell fragility test 498(7) Finish study of anemia slides and examine sternal marrow slides Find and demonstrate malaria parasites in unknown slides after study of knowns
23	240(C)-253(III) Plate VI with legend	483(f)-484(9) 495(3)-495(4)	Study demonstrations of normal white cells Find and demonstrate to instructor each type on smears prepared Do differential count on own blood and total and differential white count on unknowns Start differential counting on slides illustrating neutrophilia eosinophilia lymphocytosis infectious mononucleosis, toxic neutrophils etc which are given out as unknowns This work is to be completed by end of period 25
24	253(III)-267	379(15)-379(16) 417(4)-418(5) 507-510(B)	Study demonstrations of each type of white cells Find and demonstrate to instructor each type of white cell in leukemia slides given out By end of period 28 report differential counts on each of these unknown slides Do sedimentation rates 501(10)

* All figures and letters in parentheses refer to paragraphs All other figures refer to pages

SCHEDULE OF ASSIGNMENTS *—(Continued)

Day	For quiz period		For laboratory period
25	268-279 Review Chapters VII and VIII Plate VII with legend	418(5)-420(6) 501(10)-506 510(B)-512	Do peroxidase stain 495(3) Continue work on unknown leukemia slides Demonstrations of Paul and Bunnell test 512(4) moist cover slip preparation of sickle cell anemia and supra vital preparation 497(6) Do blood typing 510(B)-512(2) Do sulfanilamide or sulfapyridine determination 417(4)
26	280-289 Review Chapters VII and VIII	513-516	Do bleeding and clotting times clot retraction platelet count capillary resistance and prothrombin time 503(11)-506 Do quantitative vitamin C determination 418(5)
27	290-302(II)	517-523 Review Section IX	Examine sputum specimens 513-516 and ascitic or pleural fluid 522(B)-523 Review bacteriologic methods 507-510(B)
28	302(II)-313	414(G)-417(4) 420(6)-422	Examine spinal fluids 517-522(B) Do serum bromide determination 414(G) Finish study of leukemia and sternal marrow slides
29	314-324 524-526	Review sections XI XII and XIII	Do carbon monoxide and methemoglobin determinations 415(2)-417(4) Do blood alcohol determinations in groups 420(6) Demonstration of Friedman test 525(C)
30	Review using Index by Diseases as outline A-D		Practical examination in laboratory methods 327-526 Series of unknowns
31	Index by Diseases D-M		
32	Index by Diseases M-Pregnancy		
33	Index by Diseases Pregnancy-End		

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* All reagents and standards are purchasable from The Shaw Surgical Company
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